THE EFFECTS OF NICOTINE ON ANTINOCICEPTION IN MALE AND FEMALE SPRAGUE-DAWLEY AND LONG-EVANS RATS WITH AND WITHOUT STRESS

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Key Words : Rats Pain Nicotine Nociception Males Females Sprague-Dawley Long-Evans

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by

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DEDICATION

This thesis represents a collaborative effort among the three disciplines of Nursing, Medical Psychology, and Neuroscience. It is my hope that this project will be one of many collaborative efforts between the Graduate School of Nursing and other scientific disciplines. I thank my committee chair, Dr. Jane McCarthy, my major advisor, Dr. Neil Grunberg, and committee member, Dr. Eugene Levine.

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Finally, I dedicate this thesis to my dad. I only wish that he were here to read it. I think that he would be proud of it...and me.

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CHAPTER I: INTRODUCTION

Background

Cigarette smoking is a significant health hazard in the United States and in much of the world. Currently, 25% of all Americans smoke cigarettes (US Department of Health & Human Services (USDHHS), 1989; CDC, 1996). The cost in terms of human life is staggering: Approximately 3 million people die each year from smoking-related illnesses of which 400,000 are Americans (Lynch & Bonnie, 1994). People who smoke are twice as likely to die prematurely as compared with non-smokers (USDHHS, 1994).

The reasons that people initiate smoking and continue to smoke are complex. There are social, psychologic, behavioral, and pharmacologic variables that affect people s tobacco use. To prevent people from initiating smoking and to help people who already smoke to abstain, it is important to determine the mechanisms underlying the effects of tobacco.

Although there are over 4,000 chemicals in tobacco, the pharmacologically active substance in tobacco is nicotine and it is the key pharmacologic agent associated with tobacco addiction (USDHHS, 1988). Understanding the biologic effects of nicotine is critical to our understanding of smoking and its reinforcing effects.

Nicotine is known to modulate a number of psychological and biological effects including pain perception. It was observed by Mildenhall (1921) over 75 years ago that cigarette smoking had an effect on pain perception in humans. Since then, several investigators have established in animals (Sahley & Berntson, 1979) and in humans (B ttig, 1980) that nicotine has antinociceptive effects. An in-depth discussion of this

literature is provided in Chapter Three. This effect probably is mediated by nicotinic cholinergic receptors (nAChr), but it is not known which subset of nicotinic cholinergic receptors are responsible for nicotine s analgesic effect. It is also unclear if these effects are centrally mediated, peripherally mediated, or both.

Nicotine and other antinociceptive drugs including opioids are self-administered and have addictive liability. To date, most investigators have focused on a biologic addiction/reward model as the primary mechanism of addiction for nicotine and opiates. This model proposes that these drugs cause an increase in dopamine release from the nucleus accumbens which then binds to receptors upstream in the ventral tegmental region (A10) (Bozarth, 1994; Koob, 1992). These binding events are thought to produce positive reinforcement to the individual. In addition to this dopaminergic reward model for addiction, it has been suggested that other reinforcing effects play a role in nicotine addiction. These other effects include stress modulation, affect modulation, and improvement in attention and perhaps memory (USDHHS, 1988). An additional effect that may add to nicotine s rewarding effects and addiction liability is the antinociceptive/analgesic effect. Although this mechanism has not been previously proposed as a reason for nicotine self-administration, it follows logically from what is known about nicotine s antinociceptive effects and, therefore, deserves research attention.

People smoke for a variety of reasons. Perhaps some people self-administer nicotine to obtain analgesia. Nicotine s analgesic properties may help attenuate chronic or acute psychologic or somatic pains. There are a number of chronic diseases with concomitant painful symptoms (e.g., fibromyalgia, rheumatoid arthritis, degenerative

joint disease) that may induce people to smoke to alleviate pain. If this is true, then nicotine self-administration may increase to attenuate the unpleasant experiences of pain. This negative reinforcement model may play a role in nicotine reward. Therefore, nicotine s ability to produce analgesia may partially account for its addictive liability. By examining responses to nicotine-induced analgesia, it may be possible to begin to unravel additional mechanisms of nicotine s addictive properties.

The fact that nicotine possesses analgesic properties also has important implications for nurse anesthesia practice and nursing. In anesthesia, analgesic dosages are primarily calculated based on age, weight, and coexisting disease. If nicotine possesses analgesic properties, then smokers may require less anesthesia intraoperatively and less analgesia postoperatively. A heavy smoker may have more modest anesthesia requirements. If these individuals were medicated with the routine dosages of opioid analgesic, then they could be more susceptible to an overdose. In addition, this same mechanism may lead to delayed emergence from anesthesia as well. Further, patients who are acutely withdrawn from nicotine, as is done as a routine preoperative precaution, may then become hyperalgesic as a result of the withdrawal state caused by nicotine abstinence. Smokers in withdrawal may require more anesthesia and analgesia than a patient undergoing a similar procedure who was allowed to smoke preoperatively. These possibilities are particularly relevant to military medicine because one third of all military members use tobacco products. Many of these soldiers, sailors, and marines are addicted to nicotine. In an attempt to reduce morbidity and mortality associated with tobacco use, the Department of Defense (DOD) has been aggressively promoting tobacco

deglamorization and smoking cessation programs in an attempt to reduce tobacco use in the military. In addition, DOD is raising the price of tobacco products sold in military exchanges and commissaries because of the recognition of the high prevalence of smoking in the military.

These new policies also may have an unexpected impact in wartime. In the past, wounded soldiers on the battlefield had ready access to cigarettes and other tobacco products. Not only was smoking allowed on the battlefield, it was encouraged. Cigarettes were once dispensed in a package along with food rations. Although cigarettes are no longer packaged with food rations, smoking has been an accepted practice in the military until quite recently.

The battlefield of the future may be quite different. Military members may encounter a tobaccoless battlefield in the future. In light of the effects of nicotine as an analgesic, present and future troops may experience greater chronic and acute pain than did previous troops who self-administered nicotine. Acute battle injuries may require greater amounts of other analgesic agents and soldiers needing surgical procedures may require greater amounts of anesthesia and analgesia requirements than in previous conflicts. Military medics, nurses, nurse anesthetists, and anesthesiologists may be treating troops who, as a result of being nicotine-free, need and respond differently to analgesic agents.

Not only are nicotine s biologic effects relevant to the military professional, but they are also relevant to the health care professional. Even though as health care providers we should be intimately aware of the deleterious consequences that smoking

has on health, we continue to abuse tobacco products as well. A study by Nelson and colleagues (1994) examined trends in cigarette smoking for physicians and nurses using survey data sets collected between 1974 and 1991. While smoking prevalence among physicians declined from 18.8% to 3.3% (an average annual decline rate of 1.15 percentage points), during that same period smoking prevalence among registered nurses declined from 31.7% to 18.3% (an average annual decline rate of 0.88 percentage points). Licensed practical nurses declined from 37.1% to 27.2% (an average annual decline of only 0.62 percentage points). Clearly, as military nurses we need to better understand the mechanisms involved in nicotine s reward properties in order to help our patients, our colleagues, and ourselves.

Another important issue relevant to nicotine, antinociception, and military life is stress. Stress may be defined as a process in which environmental or psychological events, called stressors, come to threaten an organism s safety and well-being (Baum, Grunberg, & Singer, 1982, p. 218). Stress is ever-present in the military environment, whether it is from standing in front of general officers as part of an official ceremony or during wartime while trying to dodge bullets and keep from getting killed. Stress elicits a general biological response in an attempt to maintain the organism s homeostatic balance. During classic fight or flight conditions, the human body responds by releasing a number of substances, including endogenous opioid peptides, corticotropin-releasing hormone, and glucocorticoids (Cannon, 1932). These biological stress markers are useful to help study stress effects in animals and humans (Baum et al., 1982). The effects of these neurohumoral agents in conjunction with the effects of the stressor itself on pain

is unclear. The interaction of these stress-related variables on nicotine-induced analgesia has not been examined, but is important to study. In the stressful miliary environment, the study of nicotine, stress and pain is particularly relevant.

Another important variable to consider regarding nicotine and pain is gender. People often respond differently to drugs, particularly psychoactive drugs. The etiology of these individual differences may, in part, be a result of individual, genetic differences. It is unknown in many cases whether these differences are pharmacokinetic differences or the result of psychological, environmental or other variables. Gender is one major, genetic variable that seems to play a role in analgesic responses. There is a considerable body of literature suggesting that men and women experience different types and different levels of pain. This gender difference is evidenced by differences in prevalence studies, analgesic use, and in disability claims (Unruh, 1996). Whether gender-specific pain responses are biologically or pharmacologically based is not currently known. Whether gender differences emerge in response to nicotine-induced analgesia with and without stress is also unknown.

The study of nicotine-induced analgesia may supply information regarding the rewarding effects of the drug as well as potential therapeutic uses. Studying nicotine-induced analgesia in humans poses a number of problems. There is difficulty finding a patient sample with comparable pain syndromes. In addition, there are ethical concerns when using non-conventional analgesics to treat pain in humans. Fortunately, many of these issues can be overcome by the use of appropriate animal models. Rodent models have proven to be a valid way to study pain (Stevens, 1992). Specifically, rat studies

have been used for many years to study nociception and antinociception because they allow for the presentation of a standardized noxious stimulus as well as greater control of extraneous variables (D Amour & Smith, 1941; Wheeler-Aceto & Cowan, 1991). Additionally, by using animals with known genetic profiles, one is able to address questions of genetically-induced antinociceptive differences.

The use of tobacco products poses a serious threat to health and well-being. Even though American consumers are constantly bombarded by the media and are told how deleterious the effects are smoking are, a significant number of people continues to smoke. It has become widely known that the tobacco companies knew how addicting cigarette smoking was and used that information to enhance the sales of their products (Slade, Bero, Hanauer, Barnes, & Glanz, 1995). Paradoxically, though faced with incontrovertible evidence, people continue to smoke.

These data support the fact that nicotine is a potent drug with powerful biologic and psychologic effects. One of the effects, the ability to produce analgesia, may be important for reward reasons. Pain modulation may be one reason that people self-administer nicotine. If this is true, then the conditions under which nicotine is analgesic must be examined to better understand its reinforcing effects. Understanding nicotine s rewarding effects will assist in the efforts to help people quit smoking.

The ability of nicotine to act as an antinociceptive, under what conditions, and for which populations has important implications for military nurse anesthetists. Do smokers require less or more analgesia and anesthesia? Does stress modulate the effect? Do subtle genetic differences make a difference? Do less subtle genetic differences such as

gender differences play a role? These are important questions to answer to better understand and treat nicotine addiction. This experiment is designed to answer some of these questions.

Purpose of the Study

Understanding why people smoke cigarettes may help to prevent smoking and to assist people to quit smoking. The reasons that people smoke are multifaceted and complex. Some of these reasons are psychological and others are biological. One of the biological reasons that people smoke may be nicotine s antinociceptive property. People may smoke to attenuate acute or chronic pain. If this is true, then different individuals may have greater or lesser degrees of nicotine-induced antinociception. It is not known how individual, genotypic differences affect nicotine-induced antinociception. Additionally, it is not known how environmental conditions, specifically stress, may affect nicotine s ability to provide antinociception. The purpose of this experiment is to examine the effects of genotype, gender, and stress on nicotine-induced antinociception in the rat.

Research Questions

1. Does chronic nicotine administration have an effect on antinociception, as measured by hot-plate and tail-flick latencies, in a rat model?
2. What effect does genotype have on nicotine-induced antinociception in a rat model?
3. What effect does sex have on nicotine-induced antinociception in a rat model?
4. What effect does the physical stressor of immobilization have on nicotine-induced antinociception in a rat model?

Research Hypotheses

Hypothesis 1:

Nicotine administration will increase hot-plate and tail-flick latencies in a positive dose-dependent manner (12 mg/kg/day > 6 mg/kg/day > 0 mg/kg/day).

Rationale.: Previous studies have determined that nicotine has some antinociceptive properties in rats (Yang, Wu, & Zbuzek, 1992). Previous studies have reported that administration of nicotine dihydrochloride via osmotic minipumps is a particularly useful paradigm to study effects of nicotine to parallel effects of tobacco smoking (Grunberg, 1982).

Hypothesis 2:

Nicotine administration will enhance antinociception in female rats more than in male rats.

Rationale.: Female rats are more sensitive to the effects of nicotine on a number of behavioral measures (Grunberg, Winders, & Wewers, 1991).

Hypothesis 3:

Nicotine administration will have differential effects on antinociception in the two rat strains.

Rationale.: Previous research indicates that genetic influences may affect the way rodents respond to drug-induced analgesia (Belknap, Haltli, Goebel, & Lam , 1983). Further, effects of nicotine differ in albino and pigmented rats (Faraday, 1998).

Hypothesis 4:

Immobilization stress will enhance nicotine-induced antinociception.

Rationale.: Stress has been reported to induce both an opioid and non-opioid-mediated analgesia in animals and in humans (Agnati, et al., 1991). Immobilization stress is a reliable and valid stress manipulation (Raygada, Shaham, Nespor, Kant, & Grunberg, 1992).

Hypothesis 5:

Nicotine administration will decrease body weight in a positive dose-dependent manner (12 mg/kg/day > 6 mg/kg/day > 0 mg/kg/day).

Rationale.: Previous research indicates that nicotine administration decreases body weight (Grunberg, 1992).

Conceptual Framework

Over a lifespan, the pain experience affects all of us. We cope with pain in a variety of ways with variable degrees of success. Sometimes we use psychological coping methods, other times we use pharmacologic coping mechanisms, and sometimes we must employ both methods in order to cope with this unpleasant emotional and sensory experience. Nicotine may be one pharmacologic agent that is used to help cope with pain. In order to understand how this may occur, one must understand pain as well as the pharmacology of drugs used to treat pain. This section presents the conceptual framework for the study of nicotine, pain, stress, and gender. Essential components of pain theory, how painful inputs arrive at the brain, the biological implications of pain, and how drugs ameliorate pain are discussed. This information provides the theoretical framework for the present study of nicotine, pain, stress, and gender.

Nicotine, a drug self-administered by millions of individuals worldwide, has diverse pharmacologic properties, including analgesia. Although nicotine is unlike the classical opioid analgesics with regard to receptor type activated and mechanism of action, it is similar to opioids in that it possesses analgesic and addictive properties. One reason that nicotine is so widely used and abused may be because of its analgesic properties. The fact that these properties exists suggest that nicotine or nicotine-like drugs may someday be used clinically for pain control.

Pain is a universal experience which often provides an organism useful information about the internal milieu or about an external threat to homeostasis. Pain is often defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage (Bonica, 1979, p. 6). Because the pain experience has an emotional component, pain *per se*, cannot be unequivocally studied in animals and must be studied in humans. This is problematic because it is obviously unethical to inflict tissue damage and pain on human subjects and it is impossible to gather much of the requisite information about potential, underlying mechanisms of action. Accordingly, much of the research that investigates responses to the effects of tissue damage and of noxious stimuli has been done using animal models. It is unknown whether animals subjected to noxious stimuli are experiencing a concomitant emotional reaction and, because they are unable to communicate verbally, we cannot term the experience pain. As a result, Sherrington (1906) coined the term nociception to reflect pain-like behaviors that animals display in response to noxious stimuli. Receptors that signal the stimulation of noxious stimuli are called nociceptors. In the same way, relief from pain may occur in

a human population and is termed analgesia, yet when animals respond in a way indicating a diminution of noxious sensations are said to be experiencing antinociception.

To understand how nicotine may attenuate pain, it is necessary to understand how pain is communicated from the injury site to the brain where the noxious information is processed. Information about noxious stimuli is transmitted via nociceptors from the periphery to the neuraxis by way of thinly-myelinated, small-diameter A-delta fibers and unmyelinated, small diameter C-fibers. A-delta fibers carrying sharp, fast, pain information relay these signals to the spinal cord and terminate within laminae I, V, and X. C-fibers relay information about slow, burning, pain and terminate in Lamina II (substantia gelatinosa) (Jones, 1992). These afferent fibers then synapse in the cord and ascend rostrally in several important ascending pathways. These pathways traveling anterolaterally in the white matter of the cord, include: the spinothalamic, spinoreticular, and spinomesencephalic that are collectively referred to as the spinal lemniscus. Many spinothalamic tract neurons projecting to the thalamus will terminate at this point as much of pain processing occurs at the thalamic level, while other neurons in this tract will collateralize to project to higher structures, such as somatosensory cortex, including periaqueductal gray, and hypothalamus where they, in turn, project to forebrain limbic structures. Axons ascending in the spinoreticular tract terminate in the medullary and pontine reticular formations and are responsible for alerting and arousal behaviors associated with pain. Spinomesencephalic neurons emanating primarily from laminae I and IV ascend rostrally to the contralateral midbrain. These neurons are probably involved in the behavioral responses to pain.

Many theories have been advanced to try to explain the phenomenon of pain. These theories include the Specificity Theory and Pattern Theory (Sinatra, 1992). These theories are inconsistent with data gathered in the 20th Century. The theory that best fits our knowledge of pain is the Gate-Control Theory of Pain (Melzack & Wall, 1965). This theory suggests that small afferents, relaying pain information, enter the spinal cord and synapse in the superficial laminae (substantia gelatinosa). Neuronal activity in other large and small afferent fibers also can synapse here and, in doing so, modulate the pain response. Therefore, the substantia gelatinosa is hypothesized to act as a gating site where afferent pain information is modulated prior to ascending rostrally. The implications of this theory are that cognitive or biologic factors can influence pain transmission at this gating area. Drugs with central actions, may elicit analgesia by activating centrally located, descending-inhibitory pathways and, in doing so, attenuate the pain transmission. Nicotine may act to produce analgesia in this way.

Pain may be considered an early warning system. It signals that tissue damage is occurring or is about to occur. In most instances, this early warning system allows us to react appropriately to conditions that may threaten the organism s ability to maintain homeostasis. Once the organism is alerted, it can react to avoid the threat and maintain homeostatic balance. Once this signaling and response has occurred, pain is no longer necessary. Unfortunately, in certain circumstances such as pain following surgery, pain associated with malignancy, or pain accompanying the last days or weeks of life, pain is not serving a useful purpose and indeed is deleterious to health and well-being. Even though pain may be beneficial as a warning mechanism, it is no longer helpful after this

warning function has been served. Many people find acute and chronic pain distracting or debilitating and will take steps to relieve or attenuate pain. Smoking may be one way to attenuate pain and people may smoke for purposes of analgesia. If this is true, then how pain affects the body must be explored to understand how nicotine-induced analgesia affects biological systems.

If pain has a role in signaling that injury has occurred, then is there a reason to intervene and attempt to attenuate the pain experience? Certainly, there are humane reasons to deliver pain relief. Pain is considered noxious and most people would agree that pain adds an unpleasant dimension to their conscious states. However, there are other biologic consequences of pain that warrant consideration. These consequences may not be helping the individual cope with or protect the injury site; in fact some of these consequences may have the opposite effect.

There are pathophysiologic changes that occur with acute tissue injury as might be seen after a bullet wound or acute surgical trauma. Sinatra (1992) identifies four such pathophysiologic changes associated with acute tissue damage: (a) Neurohumoral alterations at the site and in regions adjacent to the injury; (b) Alterations in synaptic function and nociceptive processing with the spinal cord dorsal horn; (c) Neuroendocrine responses mediating hyperglycemia and a negative nitrogen balance; and (d) Sympathoadrenal activation resulting in an elevation of heart rate and blood pressure and a diminution of regional blood flow.

Locally, tissue injury involves a release of a series of neurohumoral substances that act to increase sensitivity in the injured area as well as areas adjacent to the injury.

This initial increase in sensitivity is termed primary hyperalgesia. A second type of increased sensitivity which has a delayed onset and an increased response to thermal stimuli is termed secondary hyperalgesia. Secondary hyperalgesia is thought to reflect both peripheral changes as well changes in the spinal cord (Woolf, 1989). As a result of these changes, pain may be perceived in many dermatomes above and below the injury site making movement and ambulation more difficult. Additionally, these changes in the spinal cord may contribute to an increased duration of pain even after the initial barrage of sensory input ceases (Cousins, 1989).

Following extensive tissue trauma, the body mounts a significant neuroendocrine response. This response involves hypothalamic stimulation and results in secretion of cortisol, glucagon, growth hormone, and catecholamines while causing an inhibition of insulin and testosterone (Kehlet, 1987). These neuroendocrine changes are characterized by an increase in catabolism and a decrease in anabolism, cause an increase in serum glucose levels, and a negative nitrogen (Kehlet, 1984). The consequences of shifting towards catabolic metabolism include: muscle wasting, prolonged convalescence, and immunocompromise as a result of decreased immunoglobulin synthesis. These processes imply that patients who are permitted to experience unabated pain are at increased risk for delayed wound healing, infection, and prolonged recovery.

Also associated with pain following injury is activation of the sympathetic nervous system. This activation occurs in a variety of systems, most notably the cardiovascular system. There is an increased cardiac chronotropic and inotropic response which causes the heart to pump faster and harder placing it at risk for a myocardial

ischemic event. In addition, increased peripheral vascular resistance causes the heart to pump against a higher pressure gradient. Pain causes cardiac stimulation as a result of direct stimulation of pre-ganglionic sympathetic nerves in the spinal cord as well as the outpouring of catecholamines from the adrenal medulla. Other effects of untreated, acute, pain include: hypoventilation, muscle spasm, and decreased intestinal motility (Cousins, 1994). These conditions are potentially serious and can result in increased morbidity and mortality. Smoking may elicit analgesia resulting in an attenuation of these effects.

Pain is an unpleasant sensory or emotional experience associated with actual or potential tissue damage (Bonica, 1979). There are ethical and biological reasons to alleviate pain. Several methodologies may be employed to accomplish this task. One can treat pain by modulating sensory input at the peripheral receptor or on its pathway to the central nervous system. Another method is to alter pain transmission at the level of the spinal cord prior to its rostral ascent to the brain. Alternatively, one can alter the way the patient processes or perceives the painful stimulus at the level of the brain. There are multiple sites at which pain may be attenuated and there are multiple types of therapeutic approaches designed to relieve pain.

The mainstay of the medical management of patients with pain is drug therapy, such as opioids, local anesthetics, and non-steroidal antiinflammatory drugs. One can use drugs to treat pain by: applying the drug locally to the injury site, applying drugs somewhere along the pain pathway (e.g., spinal cord), or administering the drug systemically where the drug may act at a single or multiple sites. Drugs that may be applied locally include local anesthetics. These drugs block the conduction of painful

impulses from the periphery. Another strategy is to modulate pain transmission at the level of the spinal cord with opioid agonists. Both of these techniques are useful in treating many types of pain.

Nicotine is known to have antinociceptive actions that may be mediated by central and peripheral nicotinic acetylcholine receptors. Nicotine has not been developed as an analgesic agent possibly because of its low intrinsic antinociceptive activity relative to the opioids and its side effects profile. Recently, there has been increased interest in nicotine-like compounds for the treatment of pain. Bannon and colleagues (1998) reported that an alkaloid isolated from the skin of an Ecuadorian frog, *Epipedobates tricolor,* acts as an analgesic via a nicotinic acetylcholine receptor activation. This finding has led to the development of an experimental compound that is physically similar to nicotine and may be up to 70 times as potent as morphine to provide antinociception in an animal model. This discovery emphasizes the potential value of nicotinic antinociceptive mechanisms.

Definitions

Analgesia

Absence of pain in response to a stimulus that would normally be painful (Melzack & Wall, 1965).

Antinociception

The attenuation or blocking of responses of noxious stimuli in animals (Sherrington,1906).

Nociception

The sensory phenomenon associated with unpleasant or noxious stimuli (Sherrington, 1906).

Pain

An unpleasant sensory and emotional experience associated with actual or potential tissue damage (Melzack & Wall, 1965).

Latency

The period of time that it takes to react to a given noxious stimulus.

Assumptions

1. The human experience of pain may be studied indirectly by measuring the behavioral response to a noxious stimulus in a rat model.
2. The analgesic effect that a smoker experiences is caused by nicotine alone and not by other substances found in tobacco or tobacco smoke.
3. Genotypic differences between rat strains and between sexes are analagous to individual and gender differences in humans.
4. Immobilization stress in a rat model is analogous to human stress.

Limitations

1. The noxious stimuli induced by hot-plate-and tail-flick latencies may not generalize to other noxious stimuli.
2. The use of a rodent model to measure responses to painful stimuli may not fully extrapolate to humans.

Summary

The use of tobacco products poses a serious threat to health and well-being. Pain modulation may be one reason that people self-administer nicotine. If this is true, then it is important to examine the conditions under which nicotine is analgesic. By understanding nicotine s antinociceptive effects, we may be better able to help people quit smoking. Animal models have proven to be a valuable experimental approach to examine the effects of nicotine. The present experiment examines the antinociceptive effects of nicotine in male and female rats of two different strains with and without stress.

The ability of nicotine to act as an antinociceptive, under what conditions and for which populations, has important implications for military nurses and military nurse anesthetists. Do smokers require more or less analgesia and anesthesia? Does stress modulate the effect? Do genetic differences make a difference? Does gender play a role? These are important questions to answer if we are to understand and treat nicotine addiction. This experiment is designed to provide clarification of some of these questions.

CHAPTER II: REVIEW OF LITERATURE

Introduction

This chapter reviews the relevant literature that forms the foundation for this experiment. The relevant topics discussed are: nicotine as an antinociceptive, nicotine and stress, and genetic differences to include gender differences. Nicotine is the active ingredient in tobacco that is associated with behavioral and psychological consequences relevant to addiction. Among the effects of nicotine is antinociception. Pain and nociception may be considered under the broader construct of stress. Additionally, stress is known to interact with pain. A review of this literature is critical to help understand this interaction. Finally, the way individuals respond to pain and analgesia is highly variable. Specifically, males and females report different sensitivities to a number of pain syndromes. These individual differences must be explored if we are to optimize our ability to relieve pain among different sub-populations and between genders. It is through understanding the genetic basis of antinociception that we can best provide pain relief to all people under a variety of circumstances.

Nicotine and Antinociception

As early as 1921, Mildenhall observed in an uncontrolled study that people who smoked appeared to have a greater tolerance for pain. In his studies, the method used to elicit pain was the cold pressor apparatus. This procedure involved a subject simply immersing his arm into an ice-water bath (4⁻ C) until the subject could no longer stand the pain. The subsequent analgesic or antinociceptive effect was attributed to the peripheral activation of nicotinic, cholinergic, receptors causing vasoconstriction, and

therefore, mediating a reduction in pain. This explanation was accepted until the late 1970s when renewed interest in nicotine as an analgesic emerged.

There are a number of experiments examining the effects of smoking or nicotine and antinociception. Nesbitt (1973) reported that smokers had greater endurance to electric shock than did non-smokers. This finding was replicated by Silverstein (1982). Pomerleau (1986) examined the effects of either high-nicotine cigarettes or tobacco-snuff on pain, using the cold pressor paradigm. Both high-nicotine cigarettes and tobacco snuff increased endurance to ice-water pain as compared with control subjects. In contrast, Sult and Moss (1986) reported no effect of cigarette smoking on endurance of either electric shock or cold pressor pain. A more recent series of studies by Perkins and colleagues (1994) used nicotine administered by nasal spray to determine the effects of nicotine *per se* on antinociception in smokers and non-smoking individuals. Nicotine had a significant although modest effect on increasing pain detection latencies in both smokers and non-smokers. This is an important finding because it suggests that nicotine s antinociceptive effects are not just the result of nicotine withdrawal relief as has been suggested by Hughes (1991). This experiment by Hughes is the first suggestion that nicotine has antinociceptive effects in a controlled study in smokers and non-smokers.

In 1979, this issue of nicotine as an antinociceptive was revisited by Phan, D da, Bite, and Gy rgy. These researchers used several models to assess nicotine-induced antinociception in mice. This group injected 1% acetic acid intraperitoneally and observed writhing behavior as well as using the more classic hot-plate and tail-flick methods to measure antinociception. Nicotine was administered to the mice either

intraperitoneally or intraventricularly. Nicotine had antinociceptive effects that were not blocked by central nicotinic blockade with mecamylamine, suggesting that the effects were peripherally mediated.

This question of a central versus a peripheral antinociceptive action of nicotine was further examined by Sahley and Berntson (1979). They injected nicotine subcutaneously (SC) or intracerebroventricularly (ICV) and observed the effects of peripheral (hexamethonium), central (mecamylamine), or mixed (scopolamine) cholinergic antagonist on tail-flick latencies in male Holtzman or Long-Evans rats. Subcutaneous injection of nicotine resulted in antinociception that was blocked by centrally active nicotinic or cholinergic blocking agents, but not by peripherally-acting agents. Further, small doses of nicotine (25 g) injected ICV were effective as an antinociceptive agent. These results support a central, not peripheral antinociceptive effect.

Aceto, Bagley, Dewey, Fu, and Martin (1986) went on to ask the question, Where in the CNS is nicotine acting to produce antinociception? Using [^{3} H]nicotine this group tried to correlate antinociceptive activity with site of injection, and with levels of nicotine in specific areas of the brain or spinal cord in male Sprague-Dawley rats. Although levels of [^{3}H]nicotine in spinal cord was approximately half of that found in the brain, antinociception was greatest when [^{3}H]nicotine was injected directly into the subarachnoid space. Additionally, [^{3}H]nicotine was more potent (by 40-fold) when injected subarachnoid versus the intracerebroventricular route. These data suggest that nicotine-induced antinociception is primarily mediated at the level of the spinal cord.

The above experiments all examined nicotine s antinociceptive effects after single or multiple, acute administrations. While these studies have provided broad insights into nicotine-induced antinociception, they did not attempt to address the effects over a sustained period of time which would more closely parallel the behavior of human smokers. In an attempt to address this methodologic consideration, Yang and colleagues (1992) evaluated the effects of chronic nicotine administration on antinociception. Using subcutaneously-implanted, osmotic mini-pumps, nicotine was administered over a period of 28 days to male Sprague-Dawley rats. Antinociception was evaluated by tail-flick and hot-plate latencies. Using this model of nicotine administration, these researchers found that chronic nicotine administration resulted in antinociception detected by hot-plate, but not by tail-flick. Additionally, after one week of nicotine administration the subjects became tolerant to the antinociceptive effects. This is an important study, but there is a methodological concern with regard to the independent variable. In this experiment, nicotine free base (a thick, oily substance) was administered in osmotic minipumps. Nicotine free base (as opposed to nicotine dihydrochloride or other salt forms of nicotine) diffuses poorly out of minipumps and into tissues and would result in much lower than predicted tissue nicotine levels. This concern is confirmed by the absence of significant nicotine effects on either food intake or weight gain, whereas nicotine administration to animals and humans consistently results in dose-dependent weight loss (Grunberg, 1992). Although Yang and colleagues (1992) examined the effects of chronic nicotine administration on antinociception in animals, the inappropriate use of pure nicotine makes the results of this study meaningless. The proposed experiment is designed to

correct this methodologic flaw and to expand the research design by including nicotine and stress.

Nicotine and Stress

The relationship between smoking and stress has long been recognized, but the reason for this relationship is unclear. The effects of smoking on feelings of stress are one of several complex mood state changes that accompany the use of tobacco products and cessation of their use. Some smokers report that they smoke more during periods of stress and, in fact, it has been suggested that smokers may smoke cigarettes in order to help cope with stress (Gilbert & Wesler, 1989; Shiffman & Wills, 1985). Reduction of anxiety by nicotine self-administration is a major hypothesis with regard to nicotine reinforcement.

This issue of whether nicotine attenuates stress is important. If smoking genuinely helps people reduce stress, then it may provide a beneficial health effect. This beneficial effect would have to be weighed against the deleterious health consequences of smoking when deciding whether or not to quit smoking. In addition, strategies aimed at helping people quit smoking would need to be modified to incorporate a stress reduction component.

Smokers seem to increase their smoking during periods of elevated stress, but the question is whether or not smoking helps to attenuate the psychological or biological effects of stress. Nesbitt (1973) and Silverstein (1982) reported that smokers have increased shock thresholds in proportion to the nicotine content of cigarettes smoked and attributed this increase in threshold to the anti-anxiety effects of nicotine. Although

smokers may have increased shock thresholds, it is unclear whether this effect results from nicotine s presumed anti-anxiety effect or its antinociceptive effect.

Pomerleau and colleagues (1984) reported a reduction in anticipation anxiety associated in smokers allowed to smoke while working on a difficult puzzle task. This experiment further supports the hypothesis that smoking may act as an anxiolytic. Further evidence that nicotine may play a role in the modulation of anxiety is found in a number of studies demonstrating that many people begin to smoke during early adolescence, which is a stressful period of time for most individuals (Fishburne, Abelson, & Cisin, 1979-80; Green 1979). It appears that people tend to smoke during stressful periods in their lives. When smokers attempt to quit smoking, stressful events may cause relapses (Shiffman, 1986).

Nicotine appears to have a modulatory effect on anxiety, although precisely how this effect is mediated in unknown. A number of possible mechanisms have been explored. The most likely explanation for nicotine-induced anxiolysis is activation or enhancement of a GABA-mediated mechanism, the receptors involved in benzodiazepine-mediated anxiolysis. If nicotine reduces anxiety via a GABA-ergic mechanism, then it should produce behavioral effects similar to drugs that act at the GABA receptor. This question was examined by Costall, Kelly, Naylor, and Onaivi (1989) who examined the effects of nicotine and a host of other drugs on avoidance of an aversive, illuminated white arena by rats. They found that nicotine decreased avoidance of the illuminated area and that the effects of nicotine on anxiety, in this paradigm, were similar to the effects seen after the administration of an anxiolytic agent. Conversely,

studies using the elevated X-maze anxiety test (Balfour, Graham, & Vale, 1986) or punished responding for a reward (Morrison, 1969) suggest that nicotine does not affect anxiety similar to benzodiazepine anxiolytics in rats. Though nicotine does possess anxiolytic properties, it is unclear whether these properties are mediated through GABA receptors.

Stress-related anxiety has been treated with a number of pharmacologic agents acting through serotonin (5-HT) receptors. It is possible that nicotine may be acting through serotonergic receptors. Because anxiolysis occurs through a stimulation of 5-HT_{1A} receptors (Higgins, Bradbury, Jones, & Oakley, 1988) or through antagonism of 5-HT_3 receptors (Costall, Kelly, Naylor, Onaivi, & Tyers, 1989), it is possible that nicotine may decrease stress in this way. This idea is supported by the fact that nicotine inhibits turnover of 5-HT in the brain (Benwell & Balfour, 1979). Additionally, Balfour (1991) has suggested that 5-HT may be the link between nicotine and stress reduction. While this may be a tempting explanation for nicotine-induced anxiolysis, nicotine s effects on 5-HT are restricted to the hippocampus, which receives its primary serotonergic innervation from the median raphe (Geyer et al., 1976). Further, other anxiolytic agents acting through serotonergic mechanisms appear to originate from the dorsal raphe. This discrepancy suggests that nicotine is probably not acting to attenuate stress through a serotonergic mechanism.

Nicotine s effect on anxiety is complex because not only does nicotine affect GABA and 5-HT, but nicotine also is known to affect dopamine (DA). Nicotine stimulates DA release in the mesolimbic system of the brain (Bozarth, 1994). This

increase in DA release is thought to be important in the rewarding effects of nicotine and other addictive drugs, but also may modulate stress. Data indicate that drugs that enhance dopamine secretion may facilitate the acquisition of stressful shock performance (Balfour, 1990). Because stress also has been reported to increase DA release in the mesolimbic area (Abercrombie, Keefe, DiFrischia, & Zigmond, 1989), the exposure of the individual to stress may be an important factor in nicotine self-administration. The interaction of stress, nicotine reward, and the role of dopamine warrants further examination in order to understand how nicotine may ameliorate stress responses.

Some literature seeks to support the anxiety reduction theory suggesting that nicotine, through some unknown mechanism, acts as an anxiolytic. If this hypothesis is correct, then one would expect smokers to be less anxious. While people may tend to smoke more during stressful periods, there is no convincing evidence that smoking reduces stress. In fact, the preponderance of evidence suggests precisely the opposite. Active smokers actually appear to be more anxious than non-smokers (Wheatley, 1993).

Nicotine has a modulatory effect on stress. Although it is possible that nicotine decreases stress, the finding that smokers are more anxious than their non-smoking counterparts is not consistent with an anxiety reduction model. This discrepancy may be explained by a deprivation reversal model first presented by Schachter (1978) who argued that nicotine self-administration was reinforcing, not through anxiety reduction, but rather that not smoking or insufficient nicotine intake in the smoker is anxiety producing. Therefore, the heavy smoker smokes not to become calmer, but to prevent withdrawal symptoms. It is then hypothesized that smoking during stress is an attempt to attenuate

the aversive effects associated with withdrawal. The present experiment is designed to prevent nicotine withdrawal. Animals receive nicotine continuously until the time of sacrifice. Therefore, stress caused by nicotine withdrawal should not confound the physical stressor and should not interact with measurements of pain behaviors.

There appears to be a relationship between smoking and stress. Many smokers report that smoking reduces stress, and abstinence from smoking induces a withdrawal state accompanied by stress. In either case, the full picture of how smoking and stress interact is still unclear. The first explanation may apply to some smokers, whereas the second explanation may hold true for others. The Surgeon General s 1988 report on smoking noted: The roles that individual differences in personality, temperament, and psychopathology may play in determining the nature or degree of stress-reducing effects of nicotine are yet to be determined (USDHHS, 1988, p. 407). In the present experiment a physical stressor is used to determine if stress affects measures of antinociception. If nicotine attenuates stress by preventing withdrawal, then it should not be operating in this experiment.

Nicotine and Genotype

Why do some people smoke and others do not? Why do some people sample cigarettes and never pick them up again, whereas other people become addicted almost immediately and find it difficult to stop smoking? Answers to the questions surrounding individual differences and tobacco use are important to explore if tobacco abuse is to be understood.

Why some individuals may be more vulnerable to the effects of a particular drug

and why some individuals are more likely to become addicted to drugs are complex issues. This vulnerability may arise from genetic variations or from environmental stimuli or a combination of both factors (Jones & Battjes, 1987). The observation that not all individuals are equally prone to becoming dependent upon drugs (Mann, Vingilis, Adlaf, Kijewski & De Genova, 1985) may account for the fact that certain sub-populations are more likely to abuse tobacco products than are others (CDC, 1996) as well as the fact that children who have parents who are drug-dependent are more likely to be drug-dependent themselves (Begleiter, Porjesz, Bihari, & Kissin, 1984). It is difficult to determine the impact of genotype as compared with environment and learning in these drug-dependent behaviors. Some people smoke several cigarettes and finding smoking pleasing, continue over a life-time. Others take one or two puffs and put cigarettes down, and never smoke again. Similarly, some people experience a headache, take a minor analgesic, such as acetaminophen, and are relieved. Other individuals take an equal dosage and receive little analgesic benefit. This individual variability occurs with many drugs and can only be addressed by careful experiments designed to address individual genotypic differences.

Nicotine and Gender

Genotypic differences may impact both the pharmacodynamic activity of drugs as well as their abuse potential. While some of these genotypic differences are subtle, there are major genotypic differences that need further exploration. The most robust genotypic difference is gender. Gender differences appear to play an important role in the way people respond to drugs, including nicotine (Grunberg, et al., 1991). Men and women

likely have different sensitivities to nicotine. This difference may be reflected in differences in tobacco use between the sexes. In Indonesia 75% of men smoke compared to 5% of women. In the United States, Canada and the United Kingdom, smoking prevalence currently is similar among men and women (USDHHS, 1989). While culture obviously plays a role, it is unlikely to account for such a large discrepancy. Generally, men smoke more than women and these differences appear cross-culturally (Crofton, 1990).

While it is clear that gender differences exist with regard to prevalence, tobacco product type (e.g., cigarette, cigar, chewing tobacco) (USDHHS, 1989), age of initiation (National Institute of Education [NIE], 1979), and cessation rates (Finau, Stanhope, & Prior, 1982), what is less clear is why these differences do exist. The reasons why men and women differ in response to tobacco products may be divided into two broad categories: psychosocial and biological. These two categories explain at least part of the nicotine and gender interaction. This research focuses on the biological effects of gender differences.

Nicotine appears to affect men and women differently. More women report feeling ill after smoking their initial cigarette (Silverstein, Feld, & Kozlowski, 1980). Also, women are more likely than men to report adverse reactions to smoke pollution (Shor, Williams, & Shor, 1981). Based on these observations, Silverstein and colleagues (1980) postulated that women may be more sensitive to the effects of nicotine. This hypothesis of increased sensitivity by females is supported in the animal literature. Nicotine has been shown to stimulate locomotion to a greater extent in female rodents

than in male rodents (Bowen, Eury, & Grunberg, 1986; Grunberg & Bowen, 1985). Rosencrans (1971, 1972) reported that females were more sensitive to the effects of nicotine on a number of chemical and behavioral measures when compared with male rats (after adjusting for body weight differences). Female rats also are far more sensitive to nicotine s effects on body weight and eating behavior than are males (Grunberg, Bowen, & Winders, 1986; Grunberg, Winders, & Popp, 1987).

Whereas the evidence suggests that females may be more sensitive to the effects of nicotine, there is another explanation that also fits these data. It is possible that gender differences seen in tobacco use is a function of differential metabolism. Male nonsmokers have been shown to metabolize nicotine more quickly than do female non-smokers (Beckett, Gorrod, & Jenner, 1971). This finding is supported by research reports that men who smoke cigarettes with a higher nicotine content have similar plasma levels as do females smoking low-nicotine cigarettes (Russell, Jarvis, Iyer, & Feyeraband, 1980). Further research is necessary to support a pharmacokinetic explanation.

It appears that gender differences in nicotine consumption exist. Whether pharmacodynamics or pharmacokinetics play a role in these differences, and if they do, how large a role they play, is unclear. In order to understand, treat, and prevent tobacco abuse, more information must be gathered on why males and females differ in response to nicotine-containing products and on factors contributing to this difference. It may be that males and females differ in the psychological and biological, including analgesic, effects of nicotine. Knowledge of gender differences in smoking behavior may help to prevent smoking initiation and recidivism in both genders.

Most people believe that men and women respond differently to a number of external stimuli. Pain appears to be one of these external stimuli that seems to elicit differential responses from men and women. This finding is fairly recent because early pain studies, both human and animal, used only male subjects.

Clinically, women seem to experience and report more pain than do men. Faucett, Gordon, and Levine reported that in dental pain patients, females experienced greater post-operative pain after third molar extraction than did males. Women also report a greater number of painful sites than do men (Andersson, Ejlertsson, Leden, & Rosenberg, 1993). These gender differences are not only true for acutely painful stimuli, but for chronically painful syndromes as well (Ektor-Anderson, Janzon, & Sjolund, 1993). In a recent review, Unruh (1996) examined 105 epidemiological studies of common recurrent pains for women and men. The studies reviewed involved headache, migraine, oro-facial pain, musculoskeletal pain, back pain and abdominal pain. Most of these studies reported consistent gender differences. In a majority of these studies, women reported more pain than men did, either in occurrence or intensity. Studies reveal that women experience more headache pain of both migraine and non-migraine etiologies (Honasalo, Kaprio, Heikkila, Sillanpaa, & Koskenvuo, 1993) than do men. These gender differences begin in adolescence and persist into adulthood (Balagu , Dutoit, & Waldburger, 1988).

In the laboratory it is possible to examine pain or nociception in a variety of models. Commonly, electrical, thermal, and mechanical pressure and pain have been used. However, cold pressor and ischemic pain also have been used. These stimuli vary

over a number of dimensions including intensity, temporal parameters, and quality of the painful sensation. Within the experimental literature there are a number of body areas stimulated as well as a variety of pain induction and assessment methodologies. Given this broad array of techniques and procedures, comparisons across studies are difficult. The only consistency comes from studies of mechanical-pressure pain. Using this paradigm females are consistently more sensitive than are males (Brennum, Kjeldson, Jensen, & Jensen, 1989; Buchanan & Midgley, 1987; Fischer, 1987). Fillingim and Maixner (1995) reviewed 39 laboratory studies exploring the role that gender plays in pain responses. They concluded that: (a) females exhibit greater sensitivity to laboratory pain as compared to males, (b) gender differences do not appear to be site specific, (c) gender differences appear more consistently with noxious techniques that produce deep, tonic, pain sensations (i.e. mechanical, ischemic, cold pressor), and (d) there is great interindividual variability that makes comparison among studies difficult.

With regard to nociception and pain, the literature supports the hypothesis that gender differences exist. They exist in the absence of any intervening attempts to attenuate the noxious stimulus. This issue is important if we are to understand the biological mechanisms underlying pain and pain perception. It also is important to inquire whether or not gender affects the ability to treat pain pharmacologically. It appears that gender plays a role in analgesic efficacy as well.

DeKock and Scholtes (1991) reported that female patients required significantly less self-administered, morphine sulfate via patient-controlled, analgesia pump (PCA) than did male patients after abdominal surgery. More recently, Gear, Gordon, Heller,

Paul, and Miaskowski (1996) investigated the analgesic effects of the kappa-opiate agonist pentazocine on postoperative male and female dental patients. Females receiving pentazocine had better analgesia than did males receiving similar treatment. Walker and Carmody (1993) reported that, although a single dose of ibuprofen (a non-steroidal analgesic) was an effective analgesic against electrically-induced experimental pain in male subjects, this dosage was ineffective in female subjects after adjusting for body weight. These studies suggest that gender differences in analgesic responses are substantial and that they include drugs that work through at least two different mechanisms. It seems that it is difficult to predict which gender is more sensitive to the analgesic effects of a particular drug. Whether one gender or another will report greater sensitivity depends upon the particular drug, dosage, and the means by which analgesia is measured.

The fact that humans show gender differences in response to analgesic agents is also found in the animal literature. The preponderance of the animal literature examines the effects of the mu-opioid agonist morphine in rodents. Studies in mice indicate that male mice show greater morphine-induced analgesia than do female mice (Kavaliers & Innes, 1987; Lipa & Kavaliers, 1990). These findings are supported by rat studies. Male rats receiving morphine either systemically (Baamonde, Hidalgo, & Andres-Trelles, 1989) or centrally (Kepler, Kest, Kiefel, Cooper, & Bodnar, 1989) show greater antinociception than female rats. Administration of the mu-opioid receptor agonist [D-Ala^2, MePhe^4,Gly(ol)^5]enkepkalin (DAMGO) provides similar results (Kepler, Standifer, Paul, & Kest, 1991). These findings were not supported by Ali, Sharif, and Elkadi (1995)

who reported that female rats showed significantly greater morphine-induced antinociception than did male rats. A study using the kappa opioid agonist U50,488, examining sex differences, reported that males had greater antinociception than females in response to this analgesic (Kavaliers & Innes, 1987), but the delta-opioid agonist [D-Ser^2, Leu^5]enkephalin-Thr^6 (DSLET) showed no sex differences in a rat population (Kepler et al., 1991). Findings in animal studies seem similar to human studies in that one sex does not consistently show greater sensitivity to analgesics than the other. Drug and dosage play a role in determining sex differences in animal studies of antinociception which is a similar finding in human studies.

Clearly, gender differences exist in basal pain thresholds as well as in responses to analgesic agents. This difference occurs in rodents and humans, both clinically and in a laboratory setting. It is still unknown whether this is a generalized phenomenon or only true with certain classes of drugs or with specific types of pain. These interactions between gender, pain, and antinociception merit further study.

Summary

Nicotine is an important psychotropic drug and is widely used throughout the world. It is known that nicotine is addicting, and has other effects (e.g., antinociceptive properties) that contribute to tobacco use and abuse. Many people report that they smoke more when under stress. Also, men and women differ in their use of tobacco products as well as their responses to analgesics. Therefore, the present research examines the antinociceptive effects of nicotine in male and female rats of two distinctly different strains, with and without stress.

CHAPTER III: METHODS

A timeline of the experiment appears in Table 1. The experimental design appears in Table 2. These procedures were approved by the Uniformed Services University of the Health Sciences Laboratory Animal Review Board.

Research Design and Procedures

Baseline Phase

Animals were individually gentled by 5 minutes of daily handling for three days prior to the start of the experiment. During the baseline, pre-drug, pre-stress period, measurements of body weight, hot-plate, and tail-flick were recorded. Subjects were then randomly assigned, balancing for sex and strain, to drug dosage (0 mg/kg/day, 6 mg/kg/day, or 12 mg/kg/day nicotine) and stress (stress or no stress) groups based on baseline body weight measurements to ensure that treatment groups had comparable initial body weights. This assignment resulted in 24 groups of 10 subjects per group (six groups each of Sprague-Dawley males, Sprague-Dawley females, Long-Evans males, and Long-Evans females).

Drug Administration Phase

Following the baseline period, osmotic minipumps containing either saline or nicotine were surgically implanted subcutaneously (SC) for 15 days. Before minipump implantation, animals were anesthetized with a methoxyflurane (Metophane¤, Pitman-Moore, Inc.) soaked gauze pad in a closed bell-jar. A 4 x 4 cm area between the withers was shaved, disinfected with betadine solution, and a 2 cm transverse cut was made within the disinfected area with a blunt-nosed, curved-tip, Mayo surgical scissors

Table 1.

Timeline of Experiment on Nicotine as an Antinociceptive Agent

Experimental Day	**Baseline Phase**	**Drug Administration Phase**
Days 1-3:	Animal Gentling	
Days 4-9:	Hot-Plate and Tail-Flick	
Day 15: Drug Admin. Day 1		Surgical implantation
Day 16: Drug Admin. Day 2		Stress manipulation begins (i.e.,subjects in stress cells undergo 20 min/day IM stress) Hot-Plate and Tail-Flick Time-Point 1
Day 23: Drug Admin. Day 8		Hot-Plate and Tail-Flick Time-Point 2
Day 28: Drug Admin. Day 13		Hot-Plate and Tail-Flick Time-Point 3
Sacrifice Phase Day 30: Drug Admin. 15: All subjects sacrificed		

Table 2.
Experimental Design showing Independent Variables and Breakdown by Subject

Strain (2)	**X**	**Sex (2)**	**X**	**Stress (2)**	**X**	**Drug Dosage (3)**
Long-Evans (n = 120)		Male (n = 60)		No Stress (n = 30)		0 mg/kg/day (n = 10) 6 mg nic/kg/day (n = 10) 12 mg nic/kg/day (n = 10)
				Stress (n = 30)		0 mg/kg/day (n = 10) 6 mg nic/kg/day (n = 10) 12 mg nic/kg/day (n = 10)
		Female (n = 60)		No Stress (n = 30)		0 mg/kg/day (n = 10) 6 mg nic/kg/day (n = 10) 12 mg nic/kg/day (n = 10)
				Stress (n = 30)		0 mg/kg/day (n = 10) 6 mg nic/kg/day (n = 10) 12 mg nic/kg/day (n = 10)
Sprague-Dawley (n = 120)		Male (n = 60)		No Stress (n = 30)		0 mg/kg/day (n = 10) 6 mg nic/kg/day (n = 10) 12 mg nic/kg/day (n = 10)
				Stress (n = 30)		0 mg/kg/day (n = 10) 6 mg nic/kg/day (n = 10) 12 mg nic/kg/day (n = 10)
		Female (n = 60)		No Stress (n = 30)		0 mg/kg/day (n = 10) 6 mg nic/kg/day (n = 10) 12 mg nic/kg/day (n = 10)
				Stress (n = 30)		0 mg/kg/day (n = 10) 6 mg nic/kg/day (n = 10) 12 mg nic/kg/day (n = 10)

N = 240

(Roboz¤ Surgical Instruments). The scissors were then inserted into the incision and pushed cephalad in order to make a small SC pocket. Next, a minipump containing either saline or nicotine was implanted into this pocket with the one-way release valve pointing cephalad. The incision was closed using 9 mm stainless steel clips (MikRon¤ AUTOCLIP¤, Becton Dickinson & Company) and the animal was returned to its home cage and observed for 30 minutes postoperatively to ensure healthy recovery.

Beginning on drug administration day 2 (24 hours after surgery), animals in the stress condition were placed in an immobilizer each day for 20 minutes just prior to undergoing either tail-flick or hot plate measure. Animals were randomized into stress groups based on baseline body weights and were immobilized in this way every day for the remainder of the experimental phase. For logistical purposes, the 240 rats were divided into five different groups (48 animals in each group) that were balanced for drug and stress treatments. One of the antinociceptive measures was performed on each of the groups on a single day between 0800 and 1600 hours. Each 48-subject group had minipumps implanted in a staggered fashion and the day of implantation was defined as drug administration day 1. Hot plate latencies were measured drug administration days 2, 7, and 12. Tail flick latencies were measured for all subjects on drug administration days 3, 8, and 13. These time points were chosen to measure the response to acute nicotine administration (1-2 days of drug exposure) as well as the response to longer-term nicotine administration (approximately one and two weeks of nicotine exposure). For subjects in the stress condition, hot plate and tail flick measurements began approximately 5 minutes after removal from the restraining cages. Body weight was measured and recorded throughout the drug administration phase. Subjects were

sacrificed on drug administration day 15 and specimens were collected for later analysis as part of a separate study.

Subjects

Subjects were 120 Sprague-Dawley and 120 Long-Evans male and female rats (Charles River Laboratories, Wilmington, MA). Rats were approximately 7 weeks old at the start of the experiment and weighed approximately 175 g (females) or 225 g (males). Animals were individually housed in 35.6 cm x 15.2 cm x 20.3 cm plastic cages with absorbant Pine-Dri, wood chip bedding. Animals were maintained under a 12 h reverse light/dark cycle (lights off at 0700 hours) at approximately 23 " C and 50% relative humidity. Tap water and rodent chow (Harlan Teklad 4% Mouse/Rat Diet 7001) were available continuously.

Nicotine Manipulation

Nicotine dihydrochloride dissolved in 0.9% physiologic saline or 0.9% physiologic saline (control) was administered via Alzet osmotic minipumps (Model 2002, Alza corp., Palo Alto, CA) implanted subcutaneously. Minipumps were filled with nicotine solution or saline and delivered solution at a rate of 0.48 l /hour. Dosages were 12 mg nicotine base/kg/day, 6 mg nicotine base/kg/day, or 0 mg/kg/day based on Grunberg (1982).

Stress Manipulation

Animals in the stress condition (n=120: 30 male Sprague-Dawley; 30 female Sprague-Dawley; 30 male Long-Evans; 30 female Long-Evans) were restrained in commercially available restraint cages (Centrap Cage, Fischer Scientific) for a period of 20 minutes based on Kant, Mougey ,and Meyerhoff (1989) and Raygada et al. (1992). The

restraint cage is a finger-like apparatus that restricted the animal s movements in a non-painful manner. Rats were immobilized in this manner every day during the experimental phase for a 20-minute period 0800 and 1600 hours. Animals in the non-stress condition (n=120) were left in their home cages instead of undergoing the restraint manipulation.

Measurements

Hot Plate

Hot plate latencies were measured with the Omnitech Hot Plate analgesiometer (Omnitech Electronics, Inc.). The hot plate apparatus consisted of a metal plate heated to 51" C and the apparatus was enclosed by plexiglass on all sides and top. The rat was placed on this apparatus until the rat either licked one of its hind paws or 60 seconds elapsed. When either criterion was met the rat was quickly removed and returned to its cage. The three trials were performed on each subject with approximately 7 minutes between trials. The mean of the three latencies was computed and recorded for each subject to ensure stability of measures based on Yang and colleagues (1992).

Tail-Flick

Tail Flick latencies were measured using the Omnitech Tail Flick Analgesia Monitor Model TF (Omnitech Electronics, Inc.). The tail flick apparatus was a platform with a recessed channel to hold each animal s tail. Near the end of the channel was a radiant heating coil and a photoelectric cell. Rats were placed on a platform so that the tip of the tail was extended approximately 2.5 cm beyond the radiant heating coil that was heated to 52" C. When the rat flicked its tail out of the channel, the photoelectric beam was broken, the trial ended, and the apparatus recorded the latency to respond. Three

trials were performed on each subject with trials separated by approximately 7 minutes. The mean for the three trials was computed and recorded for each subject.

Data Analysis

This experiment used a 3 (nicotine) x 2 (stress) x 2 (sex) x 2 (strain) factorial design to examine the effects of nicotine and stress on analgesia. Analysis was performed using analysis of covariance (ANCOVA) with baseline nociceptive measurements used as covariates. Pearson s product moment correlation was performed to determine if the two dependent variables had shared variance. Percent shared variance was less than 2% which justified separate ANCOVAs for each dependent variable. The Tukey Honestly Significant Difference (HSD) test was used to determine the statistical significance of differences among groups. Tukey HSD is designed to compare all possible pairs of means while maintaining the Type I error at a constant alpha level thereby providing protection against identifying too many differences as being statistically significant. Additionally, repeated-measurements analyses over time were performed. With regard to body weight measurements, male and female subjects were analyzed separately because significant body weight differences existed between the sexes at all time points and empirical literature indicates that effects of nicotine on body weight are greater in females than in males. Strains were analyzed separately because trends toward strain differences in body weight were noted at several time points. All tests were two-tailed and used an alpha level of 0.05.

CHAPTER IV: ANALYSIS OF DATA

Three subjects were dropped from all analyses: one Sprague-Dawley male (12 mg/kg/day-Stress group) and two Sprague-Dawley females (one from the 12 mg/kg/day-No stress group and one from the 6 mg/kg/day-Stress group). These subjects were dropped because of apparent surgical complications (infection or non-healing surgical site) or failure of nicotine to decrease body weight (suggesting that the minipump was not reliably delivering adequate drug dosage).

Body Weight

Figure 1 presents body weight data of male and female Sprague-Dawley and Long-Evans subjects. Nicotine administration resulted in significantly less body weight gain for all subjects regardless of strain or sex {Sprague-Dawley males [$\underline{F}$ (2, 52) = 3.394, $p < 0.05$], Sprague-Dawley females [$\underline{F}$ (2, 52) = 4.380, $p < 0.05$], Long-Evans females [$\underline{F}$ (2, 54) = 7.629, $p < 0.05$], and Long-Evans males [$\underline{F}$ (2, 54) = 2.778, $p = 0.071$]}. Based on the findings and Tukey s HSD post hoc tests: for Sprague-Dawley males and Sprague-Dawley females, 12/mg/kg/day nicotine-treated subjects weighed significantly less than saline-treated subjects; for Long-Evans females 12 mg/kg/day nicotine-treated subjects weighed significantly less than did saline-treated subjects and 6 mg/kg/day nicotine-treated subjects. These findings verified that nicotine truly was delivered to subjects as assigned to the groups. Appendix A provides means and standard errors of the mean for all body weight

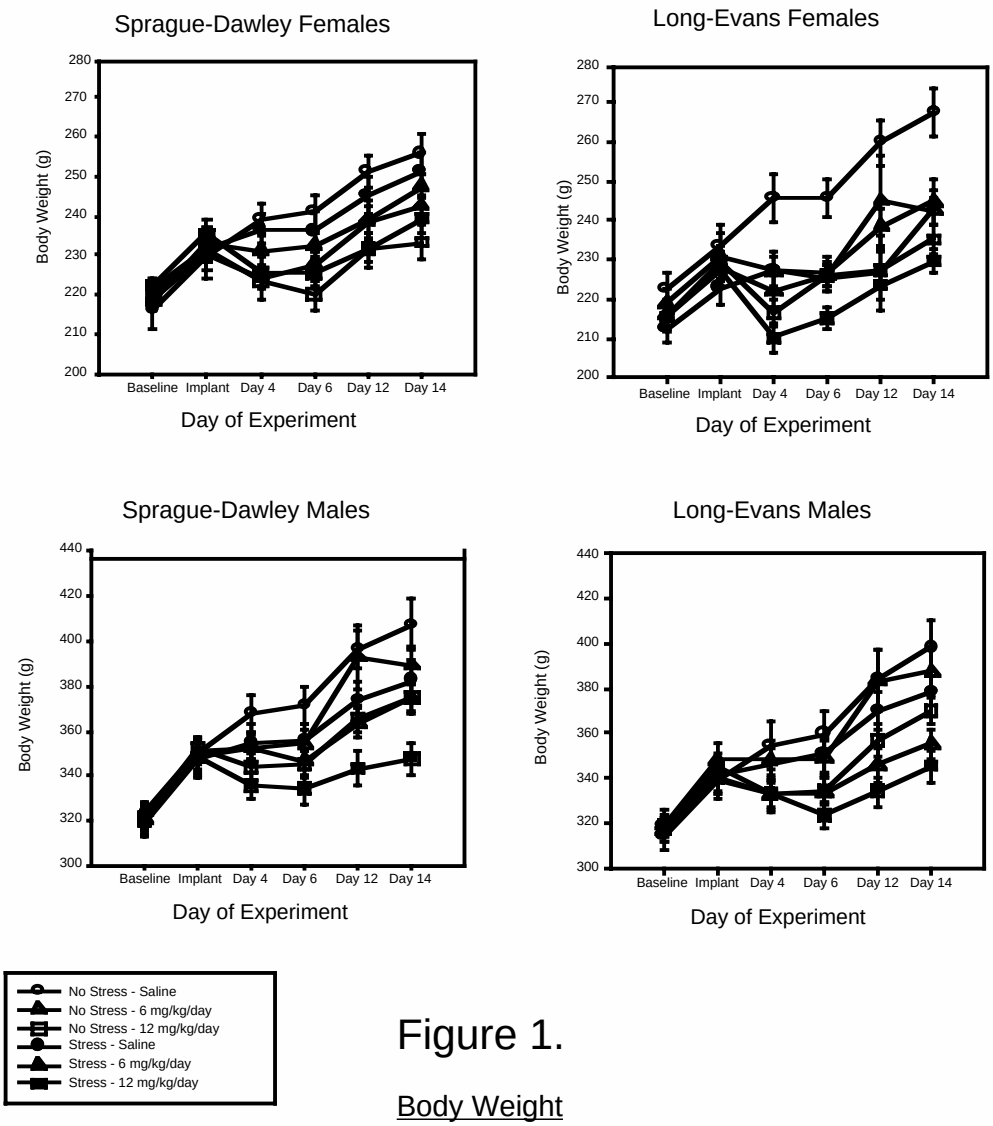

Figure 1.

Body Weight
(Mean ± SEM)

Tail Flick Latency

Figure 2 presents tail-flick latency data at time point 1 (drug administration day 3). Overall, male subjects had longer tail-flick latencies than did female subjects [$F(1,212)=9.750$, $p<0.05$] indicating that females were more reactive to the noxious heat stimulus. There also was main effect for strain such that Sprague-Dawley rats had longer tail-flick latencies than did Long-Evans rats [$F(1,104)=31.852$, $p<0.01$]. There was also a sex by strain interaction [$F(1,212)=9.634$, $p<0.05$]. Specifically, Sprague-Dawley male rats had significantly longer tail-flick latencies than did Sprague-Dawley female rats, whereas male and female Long-Evans rats had similar tail-flick latencies. At this same time-point internal analyses revealed several other significant main effects. Sprague-Dawley male rats had longer latencies than did Sprague-Dawley females [$F(1,104)=14.397$, $p<0.01$]. In addition, Sprague-Dawley male rats had longer tail-flick latencies than did Long-Evans male rats [$F(1,106)=35.690$, $p<0.01$]. There was no statistically significant difference for female rats [$F(1,105)=3.226$, $p<0.075$]. At this time-point, there were no significant drug or stress effects.

Figure 3 presents tail-flick latency at time point 2 (drug administration day 8). At this time-point there were no statistically significant findings for tail-flick latencies for drug [$F(2,212)=0.566$, n.s.], sex [$F(1,212)=1.772$, n.s.], strain [$F(1,212)=0.118$, n.s.], or stress [$F(1,212)=1.824$, n.s.].

Figure 4 presents tail-flick latency data at time point 3 (Drug Administration Day 13). Consistent with the time-point 1 findings, Sprague-Dawley rats had longer latencies than did Long-Evans rats [$F(1,212)=3.814$, $p=0.05$]. Also consistent with the time-point

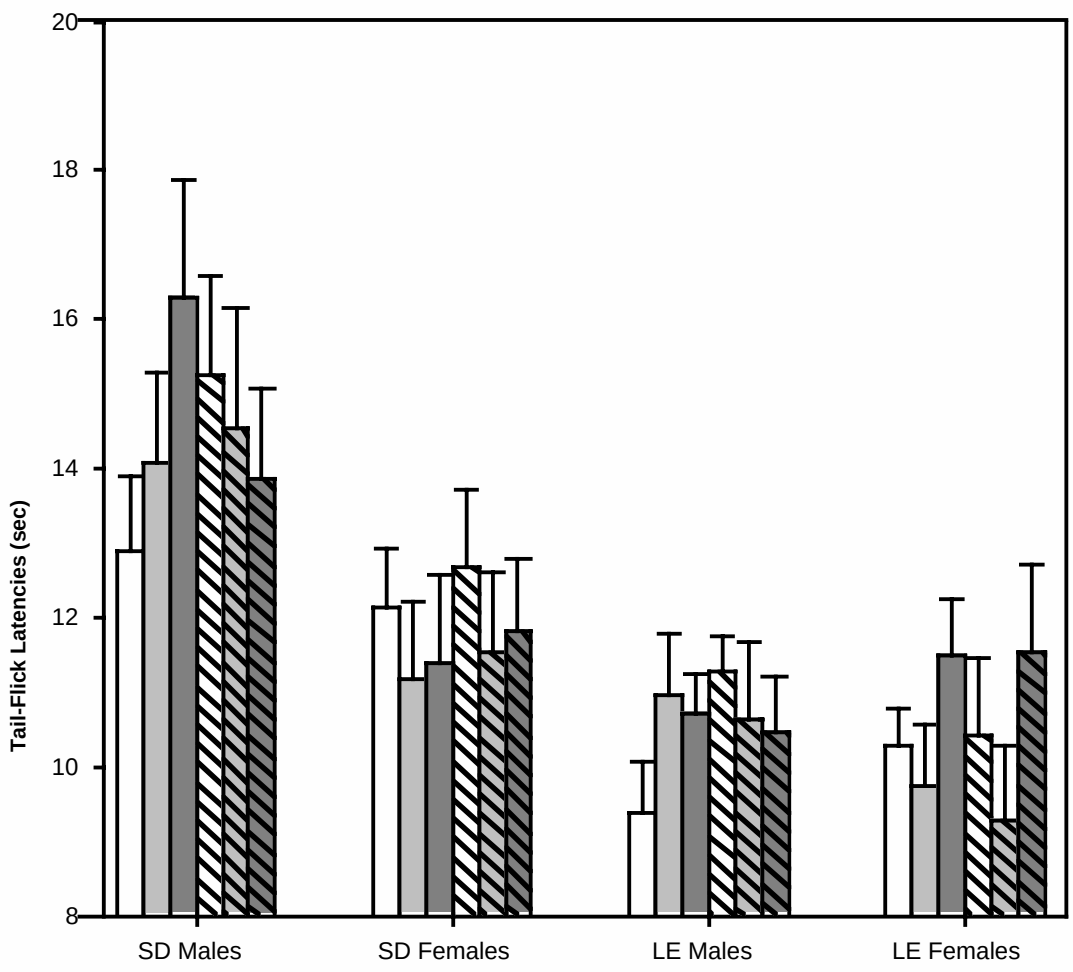

Figure 2.

Tail-Flick Latencies (Mean ± SEM)
Drug Administration Day 3

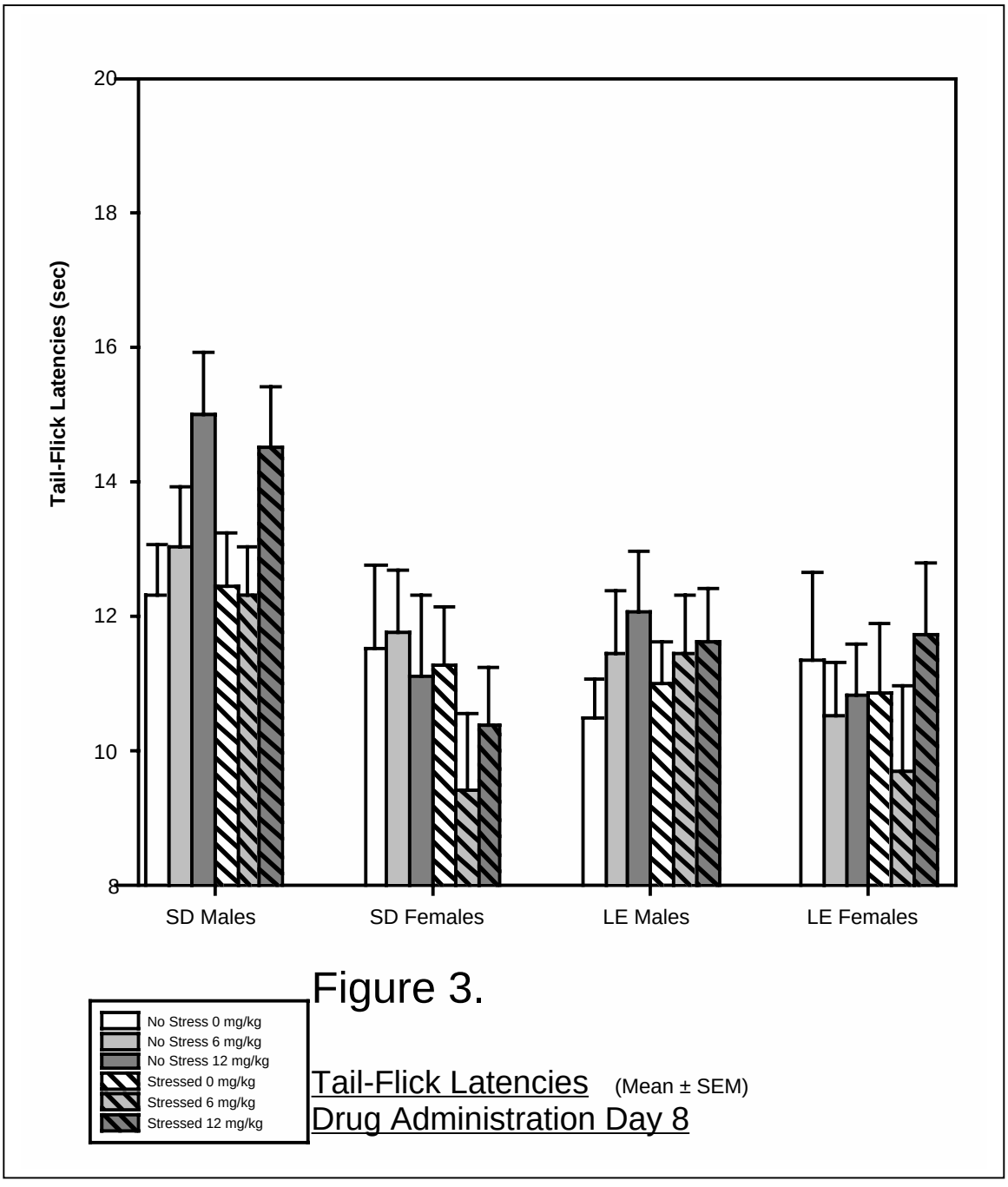

Figure 3.

Tail-Flick Latencies (Mean ± SEM)
Drug Administration Day 8

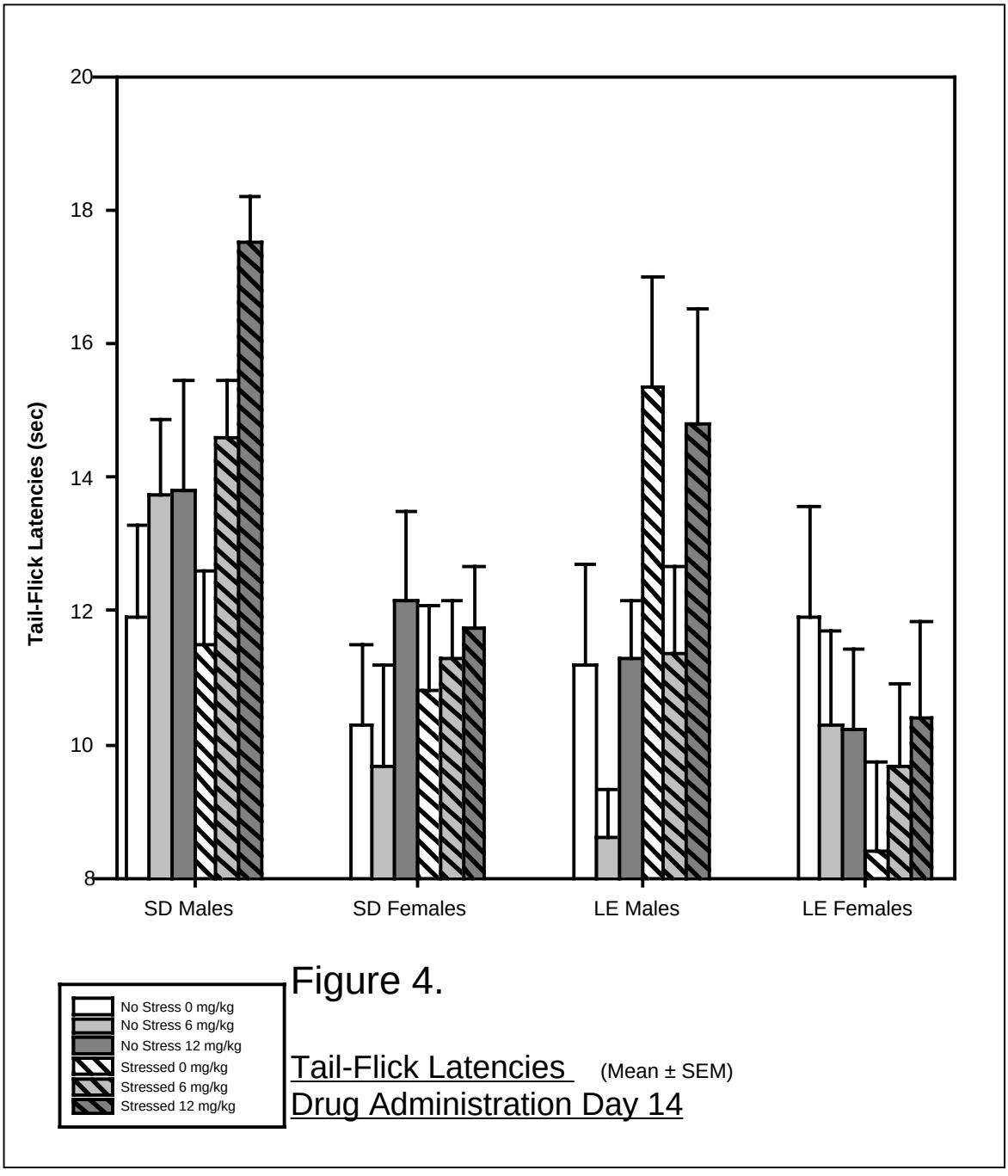

Figure 4.

Tail-Flick Latencies (Mean ± SEM)
Drug Administration Day 14

1 findings, male rats had longer latencies than did females rats [F(1,212)=18.410, p <0.01]. There was a significant main effect for drug [F(2,212)=3.198, p <0.05]. Rats receiving 12 mg/kg/day nicotine had longer latencies than did rats receiving 6 mg/kg/day (LSD p>0.05) and there was a trend for the 12 mg/kg/day rats having longer latencies than did saline controls (LSD p=0.07). There was a stress by sex interaction such that stressed, male rats had longer tail-flick latencies than did non-stressed males, whereas non-stressed, females had longer latencies than did stressed females [F(1,212)=7.016, p <0.01]. In addition, there was a strain by drug interaction such that Sprague-Dawley rats had longer tail-flick latencies in a dose-response manner, whereas Long-Evans rats displayed a U-shaped response with longer tail-flick latencies with 0 mg/kg/day and 12 mg/kg/day [F(2,212)=3.167, p <0.05].

Internal analyses revealed several other significant main effects. There was a significant main effect for drug [F(2,104)=4.731, p <0.05] and sex [F(1,104)=15.767, p <0.01] within the Sprague-Dawley strain. Sprague-Dawley rats receiving 12 mg/kg/day nicotine had longer latencies than did rats receiving 0 mg/kg/day (saline) (LSD p<0.01). Also, male Sprague-Dawleys had longer latencies than did female Sprague-Dawleys [F(1,104)=15.767, p <0.01]. For the Long-Evans strain, male rats had longer tail-flick latencies than did female rats [F(1,107)=4.076, p <0.05]. Additionally, within the Long-Evans strain there was a stress by sex interaction [F(1,107)=7.934, p <0.01]: non-stressed, female subjects had longer tail-flick latencies than did stressed, female rats, whereas for males, stressed rats had longer tail-flick latencies than did non-stressed rats. Internal analysis splitting by sex revealed significant main effects for stress

[F(1,106)=10.276, p <0.01], strain [F(1,106)=4.549, p <0.05], and drug [F(2,106)=3.514, p <0.05] only for male rats. For male rats, stress increased tail-flick latencies over non-stressed rats; Sprague-Dawley males had longer tail-flick latencies than did Long-Evans male rats; and 12 mg/kg/day nicotine significantly increased tail-flick latencies more than receiving 6 mg/kg/day did and there was a trend (p=0.073) for 12 mg/kg/day nicotine to increase latencies over 0 mg/kg/day (saline). Within sex and strain, there was a significant main effect for drug [F(2,52)=5.338, p <0.01] for Sprague-Dawley males rats receiving either 12 mg/kg/day nicotine or 6 mg/kg/day. These subjects had longer tail-flick latencies than did rats receiving saline. For Long-Evans males there were significant main effects for stress [F(1,53)=7.999, p <0.01] and drug [F(2,53)=3.451, p <0.05] such that stressed subjects had longer latencies than did unstressed subjects. With regard to drug, male, Long-Evans rats had an inverted-U response with subjects administered either saline or 12 mg/kg/day nicotine having longer tail-flick latencies than did animals receiving 6 mg/kg/day. Appendix A provides means and standard errors of the mean for all tail-flick and hot-plate latency data.

Hot-Plate Latency

Figure 5 presents hot-plate latency data at time point 1 (Drug Administration Day 3). All subjects receiving nicotine had longer latencies than subjects receiving saline [$F(2,212)=5.324$, $p<0.01$]. Specifically, subjects receiving 12 mg/kg/day nicotine had longer hot-plate latencies than did subjects receiving saline (Tukey HSD, $p < 0.05$). In addition, there was a difference in response to nicotine administration for males [$F(2,106)=3.178$, $p<0.05$] and for females [$F(2,105)=6.666$, $p<0.01$]. Male subjects receiving 12 mg/kg/day nicotine had longer hot-plate latencies than did male subjects receiving 6 mg/kg/day (LSD, $p<0.05$) and females receiving either 6 or 12 mg/kg/day had longer hot-plate latencies than did subjects receiving saline (Tukey HSD, $p<0.05$). Sprague-Dawley rats reacted to nicotine [$F(2,104)=7.307$, $p=0.01$] so that 12 mg/kg/day nicotine had longer hot-plate latencies than Sprague-Dawley rats receiving saline (Tukey HSD, $p < 0.01$). Within the Long-Evans strain there was a sex by drug interaction [$F(2,107)=3.754$, $p<0.05$] such that females responded in an inverted U-shaped response with the 6 mg/kg/day having longer latencies than saline, whereas there was not a significant change in latencies for male rats. Within sex there was a significant main effect for drug such that male rats [$F(2,106)=3.178$, $p<0.05$] receiving 12 mg/kg/day nicotine had longer hot-plate latencies than did subjects receiving 6 mg/kg/day (LSD, $p<0.05$). In contrast, female subjects responded differently to nicotine s effects [$F(2,105)=6.666$, $p<0.01$]. Females receiving either 6 or 12 mg/kg day nicotine had longer hot-plate latencies than did female rats receiving saline (Tukey HSD, $p<0.05$). Within sex and strain, there was a significant main effect for drug for Sprague-Dawley

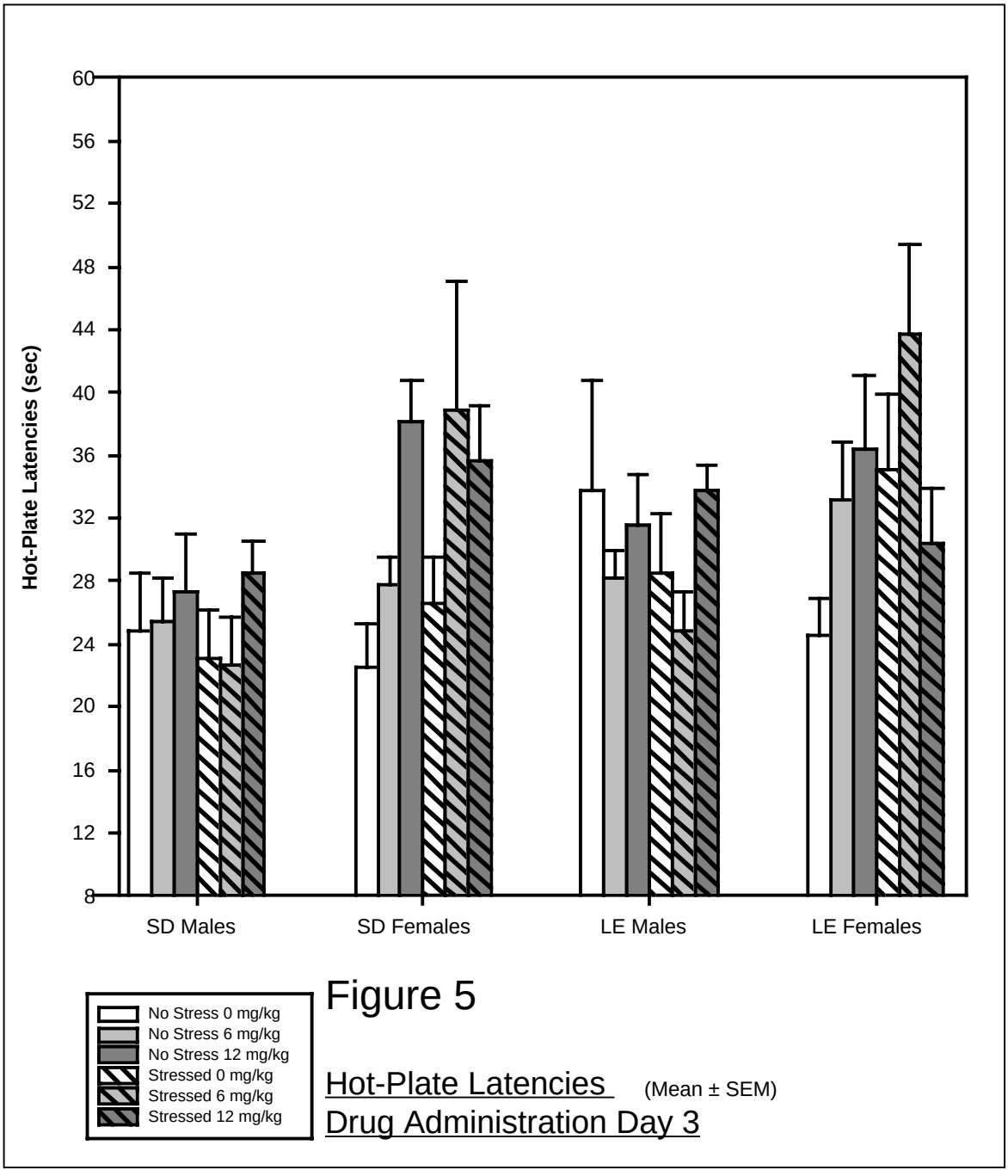

Figure 5

Hot-Plate Latencies (Mean ± SEM)
Drug Administration Day 3

male rats [F(2,52)=3.572, p <0.05] with greater hot-plate latencies in subjects receiving 12 mg/kg/day nicotine than in rats receiving saline. Also, Sprague-Dawley female rats hot-plate latencies were affected by nicotine [F(2,51)=5.239, p <0.01], so that the group receiving 12 mg/kg/day nicotine had greater hot-plate latencies than animals receiving saline (Tukey HSD, p =0.01). There also was a drug effect in Long-Evans females [F(2,53)=3.474, p <0.05], but this effect was slightly different with subjects receiving 6 mg/kg/day nicotine having longer hot-plate latencies than did subjects receiving saline (LSD, p <0.05). There were no significant effects for stress.

At time point 2 (drug administration day 7) all effects of acute nicotine administration were attenuated. There was a trend for a stress by sex interaction for Sprague-Dawley subjects such that non-stressed, male rats had somewhat greater hot-plate latencies than did stressed males, whereas stressed female rats had greater latencies than did non-stressed female subjects [F(1,104)=3.667, p =0.058]. There were no significant hot-plate findings at time-point 3 (drug administration day 12).

CHAPTER V: CONCLUSIONS

This experiment was designed to examine how nicotine affects antinociception in male and female rats of two different strains with and without stress. Although previous research has examined nicotine s antinociceptive effects in a rat model (Yang et al., 1992), potential effects of stress have not been examined. Further, previous research did not explore whether sex or other genotypic differences affects nicotine s ability to produce antinociception. It was hypothesized that nicotine would be antinociceptive for both sexes (Research Hypothesis 1) and that females would be more sensitive to these effects (Research Hypothesis 2). These hypotheses were based on findings reviewed by Grunberg and colleagues (1991) that female rats are more sensitive to the effects of nicotine on other behavioral measures. Additionally, based on previous research reporting that genotype modulated the way nicotine affected other behavioral measures in rats (Faraday, 1998), it was hypothesized that genotype would affect nicotine-induced antinociception as well (Research Hypothesis 3). It also was hypothesized that stress would enhance nicotine-induced nociception (Research Hypothesis 4) and that nicotine would decrease body weight (Research Hypothesis 5).

Research Hypothesis 1 was confirmed. Research Hypothesis 2 was disconfirmed. Research Hypothesis 3 was partially confirmed. Research Hypothesis 4 was disconfirmed. Research Hypothesis 5 was confirmed.

The results of the present experiment indicate that nicotine is a drug with antinociceptive properties. These effects, however, depended on two factors: (1) the method used to measure antinociception (tail-flick and hot-plate), and (2) the length of

time that nicotine was administered. These two independent variables are important to understand how nicotine may be analgesic under specific conditions.

Nicotine is antinociceptive, but showed differential effects on the two nociceptive measures. In the present research, the measures used to detect antinociception were the tail-flick and hot-plate latencies. These two measures were used in this experiment to determine antinociception for two reasons: (a) they are the classic tools used to measure antinociception in the rat model, but more importantly because (b) these two measures represent pain processing at two distinctly different levels in the central nervous system (Langerman, Zakowski, Piskoun, & Grant, 1995; Morgan, Sohn, & Liebeskind, 1989). The tail-flick latency is a measure of nociception that is thought to occur at the level of the spinal cord. Peripheral sensors in the rat s tail are activated by a radiant heat source causing the tail to flick reflexively away from the heat source. Therefore, higher neural centers are not involved in the processing of noxious stimuli in this paradigm. This measure is distinctly different from the hot-plate measure. With the hot-plate, an animal is placed on a heated metal plate. This noxious thermal stimulus causes a complex set of behaviors to follow. The animal must first sense the noxious heat source on it s paw, then the animal must raise the paw up off of the heat source and lick it. This reaction is considerably more complex and is considered the result of supraspinal processing.

The other variable that impacted antinociception was length of time of drug administration. Nicotine did not provide immediate analgesia at both levels of processing. At the level of the spinal cord, a full two weeks of nicotine administration was required for the animals to sense nicotine-induced antinociception. Additionally,

both sex and strain determined efficacy of the antinociception. Spinally-mediated analgesia was greater for Sprague-Dawley rats than for the Long-Evans rats. Interestingly, this effect was greater for males than for females. This finding is in contrast to prior research reporting that females are more sensitive to the effects of nicotine (Grunberg et al., 1991).

In contrast to tail-flick, subjects responded to nicotine-induced supraspinal antinociception, as measured by hot-plate latencies, markedly differently. Supraspinal antinociception occurred rapidly, after only three days of nicotine administration. Further, this antinociceptive effect dissipated and was no longer evident by the end of the first week of drug administration. For supraspinal antinociception, neither sex nor strain affected nicotine s antinociceptive effect.

The above findings indicate that both level of processing and length of nicotine administration determine the efficacy of nicotine-induced antinociception. Higher order (supraspinal) processing within the brain is sensitive earlier to nicotine s antinociceptive effects than is processing at the level of the spinal. Supraspinal nicotine-induced antinociception occurs early in the course of administration, but adapts quickly, dissipating by the end of the first week of drug administration. Conversely, spinally-mediated antinociception was not immediately evident. Two weeks of constant nicotine administration occurred before antinociception at the level of the spinal cord became evident. Additionally, genotypic differences were only seen with spinally-mediated antinociception and then only at specific time-points.

These findings suggest that the impact of sex and other genotypic differences may

operate in some instances, but not in others. These differences have implications for the human cigarette smoking population. For many smokers analgesia may occur rapidly after initiating smoking, but the effect may terminate quickly. This result would occur if nicotine induced analgesia in the brain. Therefore, the present findings suggest that supraspinal analgesia may be short-lived and that genotype and gender may have little impact. In contrast, some smokers may not experience nicotine-induced analgesia immediately, but after several weeks this analgesic effect may be perceived. This delayed-onset analgesia may be experienced by some smokers and not others. Genotype and gender differences may influence smokers perceptions of analgesia, but only after weeks of smoking, and then only for pain that is modulated at the level of the cord.

There are profound implications for these findings with regard to nicotine-induced analgesia. It is possible that brain processing of nicotine-induced analgesia is a general and widely-experienced phenomenon. Men and women of most ethnic origins may perceive analgesia acting at sites within the brain. Additionally, it may be that there is a subset of smokers that receive analgesia mediated at the level of the spinal cord, where genotype and gender may play crucial roles. For these smokers, pain relief may be enhanced. They not only perceive pain relief as a result of spinally-mediated receptor activation, but they also receive the more generic pain relief at the brain level. These smokers may smoke largely to receive an analgesic benefit.

There are clinical implications arising from the present findings. Certain pain syndromes might be amenable to analgesia mediated at brain level. Pain, such as that from a migraine headache, may be treated by a nicotine patch or nicotine injection which

would allow quick onset analgesia acting on nicotinic receptors in the brain. Many people, perhaps even most people with migraine headaches, may be able to enjoy pain relief from nicotine or nicotine analogs. In contrast, other pain syndromes, those which have pathways that are routed through the spinal cord, such as chronic low back pain, may need several weeks of nicotine administration in order to receive analgesia. In addition, males alone or only people of certain ethnicities may experience nicotine-induced analgesia. These clinical extrapolations deserve research attention. In addition, other analgesic drugs may have similar effects and these possibilities should be explored in order to provide optimal analgesia to pain patients.

The implications for nurse anesthetists treating soldiers in a field environment is clear. One third of all wounded soldiers, sailors, and marines are smokers. These individuals will come to mobile army surgical hospitals (MASH) and to combat support hospitals (CSH) with wounds requiring surgical intervention. Some of these casualties will need greater amounts of anesthesia and analgesia as a result of their smoking history. In addition, some of these military combatants who are being triaged into the delayed category will want to smoke in order to attenuate existing pain. Provisions to allow these individuals to smoke may be indicated. There is also a logistical implication for nicotine-induced analgesia. Nicotine and nicotine analogs may allow some patients to be pain-free without the need for opioids. This fact may have a huge logistical impact on patient care because unlike opioid-induced analgesia, nicotine-induced analgesia does not cause respiratory depression. As a result, these soldiers would require less nursing supervision; a limited commodity in a field environment. More patients could be safely

monitored with fewer staff. Nicotine may be an important analgesic or analgesic adjunct during war-time.

The findings of the present experiment also are important because they establish the effects of nicotine as an antinociceptive agent in a chronic nicotine administration model that has been shown to correlate with human smoking studies (Grunberg, 1982). It is noteworthy, however, that there are limitations that necessitate caution in interpreting the findings of the present experiment. Specifically, whereas tail-flick and hot-plate are useful tools to examine antinociception in animals, because they activate thermal pain receptors, they do not precisely replicate the activation of receptors when there is actual tissue damage as with an acute or chronic injury. Tissue injury involves a complex interaction of neural stimulation, the local release of inflammatory mediators, and the systemic release of neurohumoral factors. This interaction is not experienced with the two measures used in this experiment. To more precisely examine nociception and antinociception, future research should consider inflicting injury or inducing inflammation either by performing a sham surgical procedure on the animal or by injecting an irritant (e.g., carrageenan) into an animal s paw. These manipulations would stimulate nociceptive processing that more closely parallel injury-induced nociception and would provide data that are more generalizable to the physiology and pathophysiology of pain associated with tissue damage.

There is another reason to employ caution when interpreting these findings. Human pain is a complex interaction of psychological and biological factors. Psychologic factors relevant to acute and chronic pain include: developmental factors,

previous pain experience, cultural influences, focus of attention, and emotional components. For example, anxiety, fear, irritability, and frustration all can have an impact on the pain experience. Although rats are useful in the study of antinociception, it is difficult to determine whether and how human cognition and human emotion may alter responses seen in rats. This ingredient makes it difficult to relate nociception in animals to pain in humans. Even with the limitations of using a rat model of nociception to indirectly investigate the human pain experience, there is still a tremendous benefit from these techniques. These benefits include the ability to: (a) manipulate pain which would be unethical to do in a human population, and (b) investigate the biochemistry and molecular biology involved in pain by examining serum and the tissue specimens involved in these processes. Such specimens would be limited to post-mortem examinations if human subjects were used as subjects. The ability to study the brain and spinal cord directly is a valuable tool in the study of pain. These invasive studies can only be performed using animals.

Future research should continue to explore nicotine s effects on rats of other strains in order to determine whether the differences in antinociception found in the current experiment are consistent with other strains. This information is needed to explore whether the differential effects of genotype on antinociception holds true for other rat strains. The results of such studies could suggest that different populations of smokers smoke to receive analgesia, whereas other populations of smokers receive little analgesia and therefore smoke for other reasons. These experiments should extend throughout the animal s life-cycle so that antinociceptive changes associated with growth and

development might be explored. Developmental conditions may affect nociception and may help explain why individuals in certain age groups initiate smoking. Further, future research should examine the relative contributions of spinally versus supraspinally-mediated nicotine-induced analgesia. By administering nicotine directly into the cerebral spinal fluid or alternatively into a cerebral ventricle, site of action may be elucidated. Another strategy to investigate nociception may be to genetically knock-out genes for particular receptors or neurochemicals that are hypothesized to be involved in nicotine-induced antinociception (e.g., nicotinic-cholinergic receptor subtypes, acetylcholine, opioid receptors, etc.). By administering nicotine systemically to such genetically-engineered animals and measuring nicotine-induced antinociception using tail-flick and hot-plate, it may be possible to determine relative contributions from the brain and spinal cord.

Although the present research found that immobilization stress did not affect nicotine-induced antinociception in the present experiment, additional research should explore whether other stressors are important to this process. Other physical stressors, such as forced swimming, or a social stressor (e.g., crowding) may have different effects. Future research also must examine whether genotypic differences in nicotine-induced antinociception are centrally mediated or whether the behavioral differences reflect pharmacokinetic variability between different sexes and different strains of rat. It is possible that differential rates of metabolism account for some or all of the antinociceptive differences. Hepatic enzyme and drug metabolism studies should be done to determine if metabolism is responsible for genotypic differences in nicotine-induced

antinociception.

Nicotine clearly elicits analgesia in humans. Therefore, human studies should examine nicotine-induced analgesia in a clinical setting. Future research should examine anesthetic and analgesic requirements for smokers allowed to smoke preoperatively versus smokers not allowed to smoke preoperatively versus nonsmokers. The effect of nicotine and nicotine withdrawal needs to be addressed in both a surgical and chronic pain population. Patients are routinely required to abstain from smoking 24 hours prior to surgery. Whereas this rule may have a beneficial effect on the patient s pulmonary status, smoking cessation may be negatively impacting on their anesthetic by increasing their pain levels or decreasing their pain thresholds just prior to surgery. By increasing post-operative pain, the withdrawal of nicotine-induced analgesia could inhibit post-operative ambulation and perhaps delay patient discharge. If smoking cessation increases anesthesia and analgesia requirements, then it may be prudent to order another preoperative analgesic such as a nicotine patch or a potent opioid.

Summary

To synopsize, the results of this experiment suggest that people may smoke to receive analgesia. This analgesic effect may not apply to all people. It may be that specific populations of individuals are particularly sensitive to nicotine s analgesic effects. Similarly, women and men may not receive the same analgesic benefit from smoking. Men may be more sensitive to nicotine-induced analgesia. These sensitive individuals may not initiate smoking to modulate pain, but after a period of time they may find that certain aches and pains are easier to tolerate. If this is true, then smoking

recidivism in some people may be a result of increased pain and somatic discomfort. For these individuals, non-nicotine analgesics may help to prevent these people from returning to nicotine abuse.

Finally, nicotine may be an important analgesic in treating pain. Although opioids have been extensively used to ameliorate many types of pain, the use of these drugs are limited by annoying (e.g., pruritus) and sometimes life-threatening (e.g., respiratory depression) side effects. Nicotine and nicotine analogs may be useful to treat some types of pain, both acute and chronic. It may be reasonable to modify existing anesthetic regimens to include both nicotinic and non-nicotinic analgesics to attenuate surgical pain. Patients may receive slow release nicotine patches which are now used to assist people in quitting smoking to take home with them as postoperative analgesic medications. Nicotine-based analgesics may become adjuncts for many patients, depending upon the type of pain experienced and their genotypic individuality. Because the potent opioids have such a wide variety of unwanted side effects, nicotine analgesia, like the non- steroidal antiinflammatory agents, may provide patients with an analgesic alternative.

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Appendix A

Source Data for Figures

Table A1.

Source Data for Figure 1: Body Weight Data: Sprague-Dawley Males-No Stress

Time	Sex Male	Strain S-D	No Stress Saline	SEM	6 mg/kg/ day	SEM	12 mg/kg/ day	SEM
Baseline			320.2	6.8	320.3	3.9	321.4	4.3
Implant			349.6	7.3	348.4	7.5	352.4	5.0
Day 4			367.4	8.5	352.2	6.9	343.6	6.0
Day 6			371.4	8.8	354.2	7.1	345.2	5.4
Day 12			396.2	8.7	392.5	14.5	365.8	5.9
Day 14			407.3	11.2	389.1	8.7	375.0	6.5

Table A2.

Source Data for Figure 1: Body Weight Data: Sprague-Dawley Male-Stressed

Time	Sex Male	Strain S-D	Stressed Saline	SEM	6 mg/kg/ day	SEM	12 mg/kg/ day	SEM
Baseline			319.5	5.1	324.2	4.2	320.6	5.1
Implant			347.4	8.6	351.2	6.3	348.4	6.8
Day 4			355.0	8.0	352.9	5.7	336.0	5.8
Day 6			355.7	7.6	346.7	4.4	334.2	6.4
Day 12			373.8	8.7	363.6	6.1	343.3	7.5
Day 14			382.5	8.7	374.3	6.6	347.7	7.2

Table A3.

Source Data for Figure 1: Body Weight Data: Sprague-Dawley Females-No Stress

Time	Sex Female	Strain S-D	No Stress Saline	SEM	6 mg/kg/ day	SEM	12 mg/kg/ day	SEM
Baseline			216.0	4.6	218.3	2.3	219.9	2.3
Implant			229.2	4.8	233.0	4.3	231.6	3.2
Day 4			239.3	4.1	231.0	5.0	223.2	4.2
Day 6			240.9	4.4	232.6	3.3	220.0	3.7
Day 12			251.3	4.3	238.9	4.9	231.4	4.4
Day 14			255.7	5.0	247.4	3.3	233.3	4.6

Table A4.

Source Data for Figure 1: Body Weight Data: Sprague-Dawley Females-Stressed

Time	Sex Female	Strain S-D	Stressed Saline	SEM	6 mg/kg/ day	SEM	12 mg/kg/ day	SEM
Baseline			221.2	2.8	218.9	2.3	222.4	1.5
Implant			231.6	5.2	230.4	2.4	235.4	3.9
Day 4			236.7	3.7	224.1	2.4	225.7	3.8
Day 6			236.1	4.9	227.8	2.9	225.6	3.6
Day 12			245.1	5.0	238.5	4.1	231.9	3.7
Day 14			251.2	5.0	242.3	3.1	239.3	3.4

Table A5.

Source Data for Figure 1: Body Weight Data: Long-Evans Males-No Stress

Time	Sex Male	Strain L-E	No Stress Saline	SEM	6 mg/kg/ day	SEM	12 mg/kg/ day	SEM
Baseline			314.2	5.4	319.5	4.8	317.9	4.1
Implant			339.6	9.3	348.3	7.0	345.4	5.9
Day 4			354.7	10.7	348.2	8.3	332.7	5.8
Day 6			359.8	10.4	349.2	7.9	334.3	5.5
Day 12			384.3	13.3	383.6	13.6	357.3	7.0
Day 14			398.1	12.3	387.6	10.6	369.9	6.3

Table A6.

Source Data for Figure 1: Body Weight Data: Long-Evans Male-Stressed

Time	Sex Male	Strain L-E	Stressed Saline	SEM	6 mg/kg/ day	SEM	12 mg/kg/ day	SEM
Baseline			318.7	7.0	316.5	4.7	318.9	3.9
Implant			341.5	6.8	340.4	7.3	339.7	6.1
Day 4			346.2	6.7	332.9	6.6	333.3	7.9
Day 6			350.6	7.7	333.5	7.4	323.4	6.2
Day 12			370.0	7.9	345.9	8.0	334.3	6.5
Day 14			378.0	9.7	355.4	6.7	345.5	7.3

Table A7.

Source Data for Figure 1: Body Weight Data: Long-Evans Females-No Stress

Time	Sex Female	Strain L-E	No Stress Saline	SEM	6 mg/kg/ day	SEM	12 mg/kg/ day	SEM
Baseline			222.7	4.1	216.0	4.0	215.6	2.6
Implant			233.7	5.3	227.9	3.1	229.5	4.2
Day 4			245.9	6.1	221.9	3.9	216.3	3.6
Day 6			245.6	4.6	226.1	2.7	226.1	2.7
Day 12			259.8	5.7	244.9	11.9	227.5	4.7
Day 14			267.4	6.0	242.6	3.4	235.3	3.6

Table A8.

Source Data for Figure 1: Body Weight Data: Long-Evans Female-Stressed

Time	Sex Female	Strain L-E	Stressed Saline	SEM	6 mg/kg/ day	SEM	12 mg/kg/ day	SEM
Baseline			212.6	3.3	218.9	3.8	215.6	3.4
Implant			222.9	4.2	230.8	5.9	227.7	4.6
Day 4			227.1	3.8	227.2	4.9	210.0	3.5
Day 6			225.6	3.9	226.4	4.2	215.0	2.9
Day 12			226.9	9.6	238.3	4.6	223.5	3.6
Day 14			243.3	4.5	244.7	5.5	229.6	3.0

Table A9.

Source Data for Figure 2: Tail-Flick Latencies-Day 3

	No Stress Saline	SEM	No Stress 6 mg /kg/day	SEM	No Stress 12 mg /kg/day	SEM
S-D Males	12.88	1.00	14.06	1.23	16.29	1.55
S-D Females	12.12	0.80	11.17	1.03	11.39	1.16
L-E Male	9.39	0.68	10.95	0.82	10.72	0.51
L-E female	10.28	0.51	9.73	0.85	11.48	0.75

	Stress Saline	SEM	Stress 6 mg /kg/day	SEM	Stress 12 mg /kg/day	SEM
S-D males	15.25	1.32	14.51	1.63	13.84	1.21
S-D females	12.68	1.01	11.53	1.08	11.80	0.98
L-E Males	11.27	0.45	10.64	1.04	10.46	0.75
L-E Females	10.43	1.02	9.29	1.00	11.51	1.19

Table A10.

Source Data for Figure 3: Tail-Flick Latencies-Day 8

	No Stress Saline	SEM	No Stress 6 mg /kg/day	SEM	No Stress 12 mg /kg/day	SEM
S-D Males	12.31	0.77	13.03	0.90	14.99	0.96
S-D Females	11.54	1.23	11.77	0.93	11.13	1.21
L-E Males	10.48	0.60	11.46	0.92	12.09	0.89
L-E Females	11.35	1.33	10.52	0.81	10.85	0.75

	Stressed Saline	SEM	Stressed 6 mg /kg/day	SEM	Stressed 12 mg /kg/day	SEM
S-D Males	12.44	0.80	12.33	0.70	14.52	0.90
S-D Females	11.30	0.87	9.41	1.15	10.38	0.88
L-E Males	11.01	0.62	11.45	0.87	11.62	0.81
L-E Females	10.89	1.00	9.69	1.29	11.73	1.09

Table A11.

Source Data for Figure 4: Tail-Flick Latencies-Day 14

	No Stress Saline	SEM	No Stress 6 mg /kg/day	SEM	No Stress 12 mg /kg/day	SEM
S-D Males	11.91	1.39	13.74	1.13	13.80	1.65
S-D Females	10.29	1.21	9.66	1.55	12.16	1.35
L-E Males	11.19	1.51	8.61	0.71	11.30	0.86
L-E Females	11.92	1.63	10.29	1.40	10.22	1.20

	Stressed Saline	SEM	Stressed 6 mg /kg/day	SEM	Stressed 12 mg /kg/day	SEM
S-D Males	11.49	1.10	14.60	0.84	17.51	0.70
S-D Females	10.82	1.25	11.28	0.89	11.73	0.93
L-E Males	15.35	1.65	11.37	1.31	14.80	1.72
L-E Females	8.42	1.34	9.68	1.22	10.40	1.44

Table A12.

Source Data for Figure 5: Hot-Plate Latencies-Day 3

	No Stress Saline	SEM	No Stress 6 mg /kg/day	SEM	No Stress 12 mg /kg/day	SEM
S-D Males	24.91	3.57	25.39	2.77	27.34	3.69
S-D Females	22.58	2.65	27.75	1.72	38.17	2.67
L-E Males	33.81	7.00	28.17	1.73	31.58	3.20
L-E Females	24.58	2.34	33.14	3.77	36.39	4.66

	Stressed Saline	SEM	Stressed 6 mg /kg/day	SEM	Stressed 12 mg /kg/day	SEM
S-D Males	23.12	3.12	22.72	3.07	28.47	2.06
S-D Females	26.62	2.94	38.94	8.13	35.61	3.52
L-E Males	28.57	3.77	24.79	2.59	33.78	1.57
L-E Females	35.11	4.75	43.65	5.68	30.43	3.50